

Jbdt.org/ Journal articles

Volume 2, Issue 4

December , 2022

Received 15-12 -2022

Revised 17-12-2022

Accepted 20 -12-2022

MANAGEMENT OF POST-HARVEST PATHOGEN OF CITRUS SPP

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ABSTRACT

Pakistan is bestowed with an extensive variety of agro-climatic conditions, varied from tropical to temperate; allow 21 unique varieties of fruits to grow. Citrus is an important fruit within the economically important *Rutaceae* family and is cultivated in Pakistan on a 20.0461 thousand ha with production of 2.29 million tons per year. The major varieties viz. the lemon, sweet orange, kinnow, grapefruit grow in three provinces area of Pakistan, such as Baluchistan, Khyber Pakhtunkhwa, and Punjab especially in Potohar region. Despite of its fantastic capability in Pakistan, the excellent of its fruit is some distance not as good as the alternative Citrus producing nations of the world. Presently, Citrus end results are stricken by pathological troubles and its excessive deciduous is certainly one of primary thing, in the direction of reduced extraordinary after harvest.

Among these pathological elements, fungal decay causing pathogens is the principle cause of postharvest diseases of Citrus cease result after harvesting, delivery and accountable for decreasing the shelf lifestyles of Citrus which adversely have an effect on the marketplace value of fruits. Several synthetic compounds are being used to control these decay causing pathogens but these synthetic compounds reveal harmful residues on fruits that have hazardous effects on human health and environment. There is a dire need of the time to find out some alternative control methods that must be eco-friendly and safer to human health. Therefore, now the study is to assess the ability use of extracted essential oils of plants from different plant parts, chitosan and baking yeast in order to control the pathogens in-vitro and on Citrus Fruit to enhance the fruit quality and marketability.

Keywords: Citrus; *Penicillium digitatum*; Essential Oil; Chitosan; Baking Yeast;

INTRODUCTION:

Citrus is an essential and an extensively grown fruits inside the global and Pakistan is a country having high production of citrus around world. The Citrus as a genus belongs to the family *Rutaceae* is local to East Asia. Citrus is grown in subtropical and tropical location of the arena (Wu, Guohong., 2017). There are many edible species around the world in the genus citrus which are economically very important. (Reiger., 2006). Citrus, is economically critical plant life, this genus of citrus has many species and each specie has many varieties some of the

varieties of high production are; the grapefruit (*C. Paradisi*), sweet orange (*C. Sinensis*), Citrus lime (*C. Aurantifolia*), shaddock (*C. Maxima*), lemon (*C. Limon*), Kinnow (*C. Reticulata*), and citron (*C. Medica*). (S Singh., *et al* 2009). Sweet oranges mostly grow and firstly grow in sub-continent Pak-Hind, mandarins in China. Citrus fruit had been added to the new global inside during 1400s and early 1500s (Reiger, 2006). In 2019-20, international production of oranges turned into envisioned to be 47.5 million tons, led by way of Brazil, Mexico, the European Union, and China as the most important producers. (FAO., 1967).

In Pakistan, fruits and vegetables are extensively grown all over the country. Citrus is producing in all of the provinces of Pakistan, But mainly in Province Punjab five districts, Sargodha (Bhalwal), Toba Tek Singh, MandiBahauddin, Sahiwal, Khanewal, Cover total area under cultivation 60% and production of Citrus is 65%. The place and production beneath fruit increased with the aid of 22.7% and 21.1%, respectively, at some point growers produce high production of citrus around the year 2005-06, and overall vicinity beneath fruit timber by myself was 0.8 million ha (MINFAL, 2006). By MINFAL (2006) the annual production of citrus was 2.4 million ha on the area of 1.9 lac ha.

Since the creation of Kinnow mandarin (*Citrus reticulata* Blanco.), Pakistan produced 1.62 million tones citrus, which multiplied to 2.4 million in 2014 and 2015. (FAO 2016), the production of citrus is increasing as the demand of the world said by Khan in 1992. For domestic purpose and for trade citrus is an important fruit. However; its also an account of its nutritional price.

Some Synthetic fungicides has been used by growers for the first safety or prevention by pathogen or diseases, include Imazalil, thiabendazole, pyrimethanil observed by Smilanick et al., in 200), prochloraz observed by Danderson, in 1986, and guazatine discovered by FAO in 1997). Recently, researchers have proven an hobby inside the software program of non-toxic (T. Regnier). In the competition of fungicides and residual effects of fungicides, Plant extracts, inclusive of extracted essential oils, were investigated as possibility measures in opposition to pathological problem in plants (Klieber et al., 2002). Regnier et al. (2008) discovered the *In-vivo* method that change of fruit coatings using some effective oil of *Lippiascaberrima*, containing (d)-limonene, R(-)-carvone and 1,eight-cineole as vital materials (Combrinck et al., 2006). Essential oils and their additives are gaining developing interest due to their enormously at ease fame, their good sized attractiveness by using way of clients, and their effectiveness for ability multi-cause practical. (Ormancey,et al., 2001; Tripathi,et al., 2008).

ESSENTIAL OIL

Highly perishable fresh fruits and vegetables suffer much greater post-harvest losses, especially from fungal infestations, than grains and other field crops. Due to their high water content, fresh produce is easily contaminated by a wide variety of harmful fungus. Chemical management of post-harvest diseases of fruits has been the primary focus of research, and several synthetic compounds are utilized. However, many of these synthetic drugs are increasingly becoming ineffective owing to the emergence of new physiological races of pathogens (Delp 1980; Spotts & Cervantes 1986). Because of their potential carcinogenicity, teratogenicity, high and acute toxicity, protracted degradation periods, environmental pollution, and impacts on human health, the use of synthetic chemicals to manage post-harvest deterioration of food commodities is also limited (Ling 1991).

Non-toxic and action-specific compounds derived from natural sources (especially plant resources) have gained interest as a viable chemical management option in recent years. Most secondary plant compounds, including phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins, and sterols, may be found in higher plants. Various biological features of these plant compounds might be used commercially (Wain 1977; Mahadevan 1982). Biologicals are safe for the environment since they are derived from natural sources and break down quickly. Botanical pesticides, such as azadirachtin, pyrethroids, and carvone (trade name TALENT), have been effectively included into integrated pest control plans. Extensive *in vitro* investigations on artificial medium have examined the antibacterial effects of essential oils or their components on plant infections (Bishop & Thornton 1997). However, there is a dearth of studies reporting results from a comprehensive *in vivo* host-pathogen system. *In vitro* experiments cannot always be relied upon to reliably predict how a chemical will perform in the field as a fungicide. It's likely that many volatiles have effects on both the host and the pathogen. Plant products that have only been tested as antifungal agents *in vitro* are unlikely to be of interest to agrochemical companies interested in developing them as botanical pesticides.

As an antifungal agent against a wide variety of plant infections, this reference recommends using lemongrass oil (*Cymbopogon citratus* L.). Treatment with

lemongrass oil reduced the time it took for *C. herbarium*, *B. cinerea*, and *R. stolonifer* spores to germinate in suspension and during germination (Tzortzakis and Economakis 2007).

CHITOSAN

The natural biopolymer chitosan has been shown to inhibit the growth of yeasts and fungi, making it a useful tool in the fight against postharvest deterioration in fruit.

A large number of researchers cited vegetables as a source of health benefits (Xianghong and Shiping,2009 ; Abd-El-Kareem, et al.,2002). Because of its natural makeup, antibacterial action, and induction of defensive responses in plant tissue, chitosan is being considered as a viable alternative treatment for fresh produce (Cheah, et al., 1997).

A wide variety of fruits, including pears (Meng et al., 2010), strawberries (El- Gaouth et al., 1992, Morsey et al., 1999), table grapes (Granfrance et al., 2007, Ait Barka et al., 2000), tomatoes (Liu et al., 2007; Ben-shalom et al., 2003; Bdawayya and Rabeab,2009) (Jang and Liu,2001).

cucumber (Ben-Shalom,et al., 2007; Bhaskara,et al., 2000 ;) (Ben-Shalom,et al., 2007; Bhaskara,et al., 2000 ;) Fungal strains such *Penicilliumdigitatum*, *Penicilliumitalicum*, *Botrydiplodialecanidion*, and *Botrytis cinerea* were tested for their susceptibility to chitosan's antifungal properties. It was found by Hongyin et al. (2011) that chitosan is more efficient than TBZ in preventing citrus fruit deterioration brought on by *Penicillium digitatum*, *Penicillium italicum*, *Botrydiplodialecanidion*, and *Botrytis cinerea* after 14 days of storage at 25 °C (fungicide).

After seven days of incubation at 20°C, Yu et al. (2007) discovered that chitosan, alone or in combination with *Cryptococcus laurentii*, may successfully suppress the blue mold rot caused by *Penicilliumexpansum* in apple fruit.

BAKING YEAST

The Dutch word "gist," meaning "foam," was the original source for the English word "yeast." This foam develops during the fermentation process of beer wort and is a key ingredient in the final product. The French term "levure" also refers to yeast's essential function in making bread rise. When considering the cultural, economic, and scientific impact, few other microorganisms can compare to yeasts. Yeasts are used in a wide variety of businesses, including those related to medicine, biotechnology, and the pharmaceutical industry, as well as in the food and beverage fermentation process. The primary function of certain microorganisms is to compete with and eventually eliminate other microorganisms that aren't wanted, such harmful yeasts, molds, and bacteria. Hayduck was the first to recognize yeast's inhibitory function (1909). Other researchers reported the yeasts' hostile behavior against one another a little later.

involving the generation of lethal poisons called "mycocins" as a secondary metabolite (Young and Yagiu, 1978; Rosini and Cantini, 1987; Walker et al., 1995; Suzuki et al., 2001; Marquina et al., 2002). Organic acids, antibiotic factors, volatile acids, hydrogen peroxide, and other substrates are all mentioned by Viljoen (2006) as contributing to the antimicrobial activities of yeasts in fermented foods and drinks. However, determining how yeasts block has received little attention. Specifically, this research aims to: Poisoned food technique used to test the efficacy of essential oil, chitosan, and baking yeast against *Penicillium digitatum*, a fungus that may cause problems for citrus after harvest.

MATERIAL AND METHODS

Collection Of *Penicillium* Spp Culture

*Penicillium*spp isolate PdFL1 has been taken from fungal Plant Pathology Laboratory, Department of Plant Pathology, PirMehr Ali Shah Arid Agriculture University Rawalpindi for further process.

Collection of Plant Material

Seeds of *Foeniculumvulgare* (Fennel), leaves of *Tagetes erecta* (Marigold) collected from herbal store. Chitosan and *Saccharomyces cerevisiae* (Baking Yeast) obtained from the Metro stores Islamabad.

Table 1.1: Selected Agents, Scientific names, Plant parts for preparation of Essential oil and other treatments.

Selected Agents	Scientific Names	Plant Parts
Marigold Plant	<i>Tagetes erecta</i>	Leaves, Flower
Fennel Plant	<i>Foeniculum vulgare</i>	Seed
Chitosan	<i>Chitosan</i>	Powder
Baking Yeast	<i>Saccharomyces cerevisiae</i>	Powder

Preparation of Essential Oils

Leaves of *Tagetes erecta* (Marigold) and seeds of *Foeniculum vulgare* (Fennel), dried under shade for 4-5 days to attain the specified shape. By using Grinder (Pascall Motorised Pestel & Mortar, Machine No. 20069). To weigh down these botanical substances to attain high-quality powder. Then, Essential oils were extracted by using Soxhlet's equipment by adopting protocol (Lu and He, 2010). The obtained Essential oils in sterilized glass vials stored at

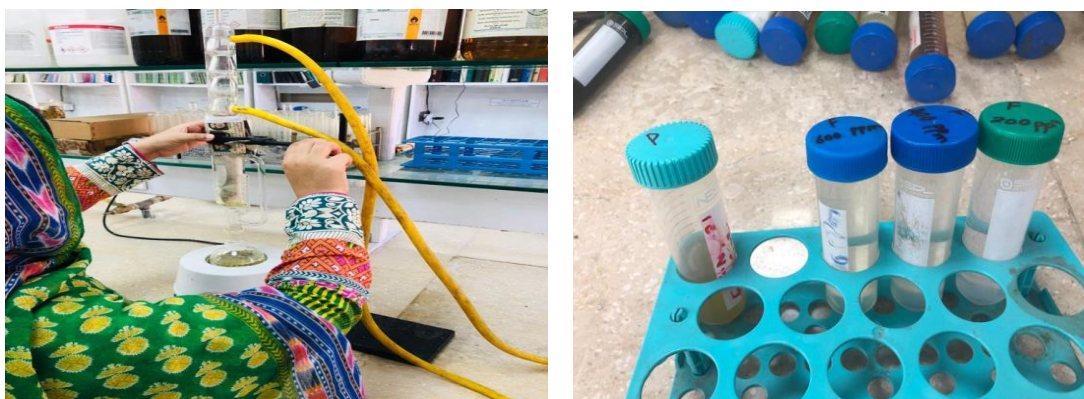


Figure 1. Preparation of Essential oils at room temperature

In-vitro screening of selected bio-agents against *penicillium digitatum*.

Poisoned food technique was used for *in vitro* evaluation of plant essential oils (EOs), Chitosan and Baking Yeast against *Penicillium digitatum* pathogen of citrus followed by (Perrucci et al., 1994; Prakash, Kedia, Mishra, & Dubey, 2015; Singh, Dikshit, Sharma, & Dixit).

Essential Oils

Two essential oils were used, (a) Marigold and (b) Fennel by preparing the three different concentrations at (200, 400, and 600PPM).

The concentrations of the critical Then dissolve the requisite quantities in 0.5 ml of 0.1% Tween 80 in Distilled Water and then mixed with 9.5 ml of Potato Dextrose Agar medium.

Chitosan

The Chitosan powder blends with in 100ml of Distilled water with different concentrations (0.2g, 0.4g, and 0.6g). Then, dissolved 0.5ml of the solution (Chitosan with Sterilized Distilled water) with different concentration in 9.5ml of Potato Dextrose Agar medium.

Baking Yeast

The Baking yeast powder blend inside the 100ml of Distilled water at three different concentrations (0.2g, 0.4g, and 0.6g).Then, dissolved 0.5ml of the solution (Baking yeast with Sterilized Distilled water) with different concentration in 9.5ml of Potato Dextrose Agar Medium.

The Mycelial Radial Growth has been observed after 7 to 10 days by using following formula:

$$\text{Mycelial Radial Growth Inhibition (MRGI \%)} = \frac{dC - dT}{dC} \times 100$$

“dc” presenting the mean diameter of control.

“dt” presenting the mean diameter of treatment.

Application Of Effective Treatment On Citrus Fruits For Postharvest

Management Of *Penicillium*Spp

After in-vitro screening of all the selected agents (Essential oils, Chitosan and Baking Yeast). Apply the most effective treatment from all the selected agents on citrus fruit for the management of *Penicilliumdigitatum*. The mature and wholesome fruits used for the test. The fruit sterilized from surface by running water with 0.1% NaOCl for 2min accompanied through washing in distilled water two times.

The trial was done by two different techniques, Curative Technique and Preventive Technique. (Wilma du Plooya,. et al 2009).

Effective Treatment from selected agents was used for its application on 9 fruits of citrus against fungal activity of *Penicilliumdigitatum* that was already in the pure form.

In preventive method, On First day, the effective selected agents was prepared in 500ml distilled water with 1% tween 80 solution and dip the wounded fruits one in each concentration of oil for 3 mins, dry it for overnight. On second day then made the inoculum of 40µl of spore suspension (10^5 spores/ml) of *Penicilliumdigitatum* and then wounded the treated fruits and dip in the spore suspension for 3 mins.

In Curative technique, The inoculum of 40µl of spore suspension (10^5 spores/ml) of *Penicilliumdigitatum* and then wounded the treated fruits and dip in the spore suspension for 3 minutes and leave it overnight for dry at 20°C. On the second day, the effective selected agents was prepared in 500ml distilled water with 1% tween 80 solution and dip the wounded fruits one in each concentration of oil for 3 mins, dry it for overnight.

Statistical Analysis

All the experiments will be replicated four time and data sets will be statistically analyzed using SPS software. The statistical level of significance will be fixed at $P \leq 0.05$. The design Complete Randomized Design two factorial will be used.

RESULTS AND DISCUSSION

Evaluation of Selected Bio-Agents against *PenicilliumDigitatum* Post-Harvest Pathogen of Citrus

Poisoned food technique was used for in vitro evaluation of two plant Essential oils (EOs) at different three concentrations (200PPM, 400PPM, and 600PPM), Chitosan at different three concentrations (0.2g, 0.4g, and 0.6g), Baking Yeast at different three concentrations (0.2g, 0.4g, and 0.6g) respectively.

Efficacy of Plant Essential Oils against *Penicilliumdigitatum*.

Essential oils extracted from Marigold (Flower and Leaves) and Fennel (Seed) by running Soxhlet Apparatus for 06 hours, stored in Refrigerator at 04 °C.

Then apply on pathogen by Poisoned Food Technique at three different concentrations and each have three replications with control. The effect of both plant essential oils on mycelial growth inhibition of *Penicilliumdigitatum*(PdFL1) tested *In-vitro* at three different concentrations (200PPM, 400PPM, and 600PPM).Mycelial radial growth of Marigold at three different concentrations (200PPM, 400PPM, and 600PPM) showing results of In-vitro screening (M1G1, M2G2, and M3G3) and Mycelial radial growth of these Treatments was (52, 51, and 0%) respectively. Mycelial radial growth of Fennel at three different concentrations (200PPM, 400PPM, and 600PPM) showing results of In-vitro screening (F1G1, F2G2, and F3G3) and Mycelial radial growth of these treatments was (84, 55, and 20%) respectively.

Table 1. Showing result of mycelial radial growth of both essential oils against *Penicillium digitatum*.

Concentration	Essential Oils				Control
	Fennel		Marigold		
200PPM	F1G1	84%	M1G1	52%	0%
400PPM	F2G2	55%	M2G2	51%	0%
600PPM	F3G3	20%	M3G3	0%	0%

From all the three different concentrations of both essential oils (Fennel and Marigold) (200PPM, 400PPM, and 600PPM).600PPM of Marigold showing effective results against *Penicilliumdigitatum*. There’s no growth of pathogen at 600PPM concentration of Marigold. The 600ppm would be the one of effective treatment from all the treatments, because it retarding the growth of pathogen of *Penicilliumdigitatum*. The 600ppm concentration plate is showing by M3G3. In this M is representing the Marigold and G is a term to differentiate according to the method.

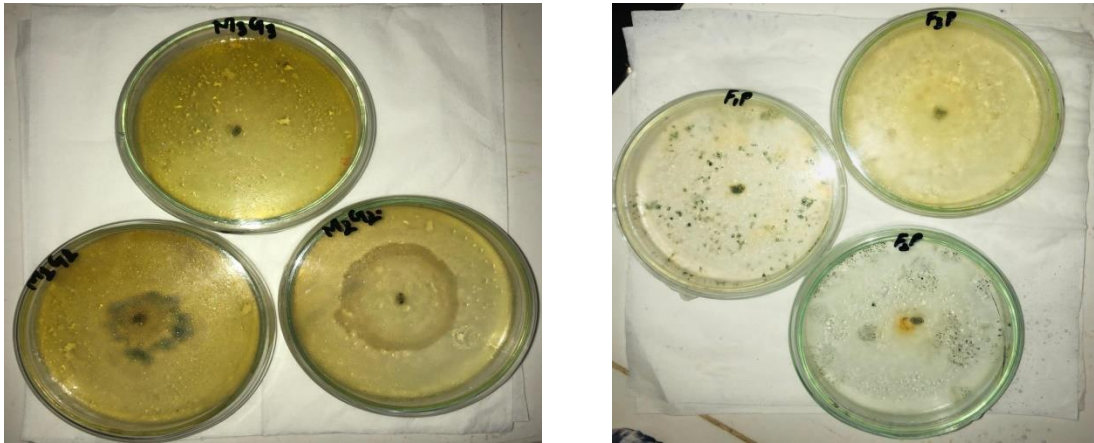


Figure2. In-vitro screening of Marigold and Fennel Essential oil results against *Penicilliumdigitatum*

Efficacy of Baking Yeast against *Penicilliumdigitatum*.

Powder of Baking Yeast purchased from market. Then apply on pathogen by Poisoned Food Technique at three different concentrations (0.2g, 0.4, and 0.6g) in 100ml with control.

The effect of Baking Yeast on mycelial growth inhibition of *Penicilliumdigitatum*(PdFL1) tested *In-vitro* at three different concentrations (0.2g, 0.4, and 0.6g) in 100ml (Y1G1, Y2G2, and Y3G3) and Mycelial radial growth of these Treatments were (72, 65, and 45%) respectively.

Table 1. Showing result of mycelial radial growth of Baking Yeast against *Penicilliumdigitatum*.

Concentration in 100ml	Essential Oils	Control
0.2g	Y1G1	72%
0.4g	Y2G2	65%
0.6g	Y3G3	45%

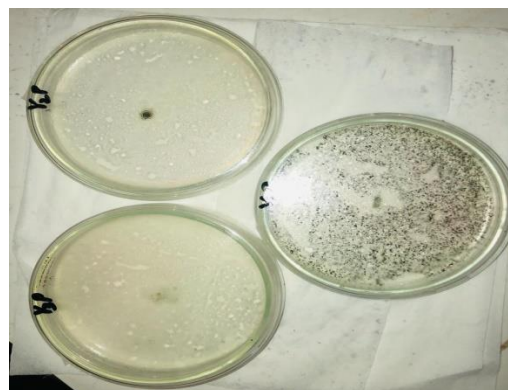


Figure3. *In-vitro* Baking Yeast results against *Penicilliumdigitatum*

Efficacy of Chitosan against *Penicilliumdigitatum*

Powder of Chitosan purchased from market. Then apply on pathogen by Poisoned Food Technique at three different concentrations (0.2g, 0.4, and 0.6g) in 100ml with control.

The effect of Chitosan on mycelial growth inhibition of *Penicilliumdigitatum*(PdFL1) tested *In-vitro* at three different concentrations (0.2g, 0.4, and 0.6g) in 100ml (C1G1, C2G2, and C3G3) and Mycelial radial growth of these Treatments were (77.7, 64.5, and 53.4%) respectively.

Table 2. Showing result of Mycelial radial growth of Chitosan against *Penicilliumdigitatum*.

Concentration in 100ml	Essential Oils		Control
0.2g	C1G1	77.7%	0%
0.4g	C2G2	64.5%	0%
0.6g	C3G3	53.4%	0%

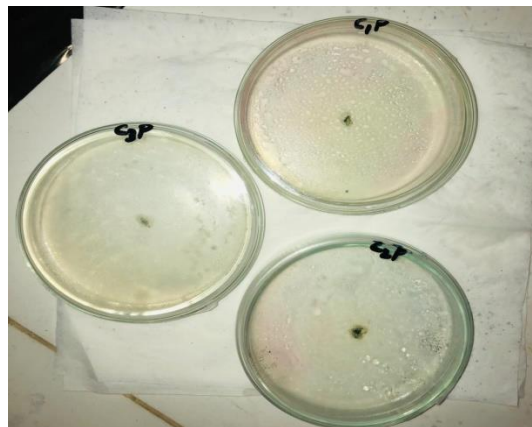


Figure 0. *In-vitro* Chitosan results against *Penicilliumdigitatum*

From all the three selected agents (Essential oils, Chitosan, Baking Yeast) different concentrations of both essential oils (Fennel and Marigold) (200PPM, 400PPM, and 600PPM). Chitosan at different concentration (0.2g, 0.4g, and 0.6g) and Baking Yeast at different concentration (0.2g, 0.4g, and 0.6g). 600PPM of Marigold showing effective results against *Penicilliumdigitatum*. There's no growth of pathogen at 600PPM concentration of Marigold.

Application of Effective Treatment On Citrus Against *PenicilliumDigitatum* Post-Harvest Fungal Pathogen Of Citrus

The effective treatment from selected agents (Essential Oils, Chitosan and Baking Yeast) by Poisoned Food Technique for the management of *Penicilliumdigitatum* was Marigold Essential oil.

Apply the most effective treatment (Marigold Essential Oil) from all the selected agents on citrus fruit for the management of *Penicilliumdigitatum*.



Figure5. Dipping of fruits in Inoculum (*Penicilliumdigitatum*) for 03 minute and Treatment of Marigold Essential oil)

The mature and wholesome fruits used for the test. The fruit sterilized from surface by running water with 0.1% NaOCl for 2min accompanied through washing in distilled water two times. The trial was done by two different techniques, (a) Curative Technique and (b) Preventive Technique. (Wilma du Plooya, et al 2009).

Effective Treatment from selected agents was used for its application on 9 fruits of citrus against fungal activity of *Penicilliumdigitatum* that was already in the pure form.

Preventive Method

In preventive method, on First day, the effective selected agents (Marigold E.O) was prepared in 500ml distilled water with 1% tween 80 solution and dip the wounded fruits in three different concentrations (200PPM, 400PPM, and 600PPM) of Essential oil for 3 mins, dry it for

overnight. On second day, the E.O treated fruits dip in spore suspension(10^5 spores/ml) of *Penicilliumdigitatum* for 3 mins.

Decaying results of citrus fruits after the 1st day at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (0%, 0%, and 0%) respectively.

Whereas, on 7th day decaying results was recorded at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (5%, 2%, and 0%) respectively. Whereas, on 14th day decaying results was recorded at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (14%, 9%, and 0%) respectively.

Table 3. In Preventive Method, Decaying of citrus fruit after applying (Treatment and Pathogen) on 1st day, 7th day and 14th day.

Days	Concentration in 100ml	Essential Oil	Control
Day 01	200ppm	C1M1	0%
	400ppm	C2M2	0%
	600ppm	C3M3	0%
Day 07	200ppm	C1M1	5%
	400ppm	C2M2	2%
	600ppm	C3M3	0%
Day 14	200ppm	C1M1	14%
	400ppm	C2M2	9%
	600ppm	C3M3	0%



Treatment

Figure 6. Preventive Method, Application of Effective 01,07 & 10 Day)

Curative Method

In Curative method, dip the sterilized wounded citrus fruits in spore suspension (10^5 spores/ml) of *Penicillium digitatum* for 3 mins and leave the fruits for overnight. On second day, Pathogen treated fruits diipp in the three different concentration of effective agent (Marigold E.O) for 3 mins.

Decaying results of citrus fruits after the 1st day at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (0%, 0%, and 0%) respectively. Whereas, on 7th day decaying results was recorded at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (8%, 5%, and 0%) respectively. Whereas, on 14th day decaying results was recorded at room temperature on three different concentration (200PPM, 400PPM, and 600PPM) shows (22%, 16%, and 4%) respectively.

Table 4. In Curative Method, Decaying of citrus fruit after applying (Treatment and Pathogen) on 1st day, 7th day and 14th day

Days	Concentration in 100ml		Essential Oil	Control
Day 01	200ppm	C1M1	0%	0%
	400ppm	C2M2	0%	0%
	600ppm	C3M3	0%	0%
Day 07	200ppm	C1M1	8%	0%
	400ppm	C2M2	5%	0%
	600ppm	C3M3	0%	0%
Day 14	200ppm	C1M1	22%	0%
	400ppm	C2M2	16%	0%
	600ppm	C3M3	4%	0%

Data was collected at different interval of date in Curative method, after 01st Day, 07th Day, and 14th Day. Data showing the decaying of fruits and effectiveness of Treatment against decaying of pathogen on citrus fruits. As concentration increase decaying by pathogen decrease.



Figure 7. Curative method Application of Effective treatment (01st, 07th, 14th Day)

CONCLUSION

Penicilliumdigitatum observed as a destructive post-harvest fungal pathogen of citrus causing mold on fruit. From all the selected agents (Essential oils, Chitosan and Baking Yeast) the Marigold E.O observed as best treatment against *Penicilliumdigitatum* on citrus.

Marigold E.O with concentration 600PPPM ranked best as anti-fungal compound against *Penicilliumdigitatum* on citrus fruit. This natural fungicide shows promise and may be suggested for use against additional citrus rot diseases. Additionally, it may be utilized to extend the freshness of other citrus kinds.

These are preliminary studies on the effectiveness of Marigold Essential Oil. The product's effectiveness, activity spectrum, and the estimated cost of its usage can only be determined by continuous field evaluation.

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