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Extracts Some Plants on Controlling Green Mold of Orange and on Postharvest Quality Parameters

Ghassan F. Al-Samarrai, Harbant Singh and Mohamed Syarhabil

School of Bioprocess Engineering, University Malaysia Perlis (UinMAP),
Kompleks Pusat Pengajian Jejawi 3, 02600 Arau, Perlis, Malaysia

Abstract: Ten Crudes plant extracts of Neem, Pong-pong, Chilly, Lemon grass, Tumeric, Clove, Green chirayta, Mahogani, Curry leaf and Ginger were test against *Penicillium digitatum*, the causes of green mould disease of orange fruits, was evaluated for their inhibitory effect in *vitro* and in *vivo* (500 to 3000ppm) during storage conditions(2000 to 5000 ppm). Followed to lethality test LC50 (BST) to determine the lethal dose. The concentration at 3000 ppm from Neem, Pong-pong and Chilly showed the highest inhibition growth reached the (90%) followed by Lemon grass and Ginger by inhibition reached (70%). *In vivo* experiments, treating fruits 21 days crude extraction from Neem, Chilly and Pong-pong at conc. of 4000 and 5000ppm showed significantly reduced the disease severity of fruits (100%) compared with untreated fruits (control) at 25°C, through significantly reduced the undesirable fruits percentage, fruit weight loss percentage during storage for 21 days. Lethal concentration (LC50) values of the crude extracts at 20, 5 and 30 $\mu g/ml^{-1}$ from Neem, Pong-pong and Chilly showed very high lethal toxicity on brine and effect Lemon grass and Ginger in killed 50% was at 495 and 473 $\mu g/ml^{-1}$ respectively. Plant extracts or botanicals have a bright future in modern plant protection to replace conventional synthetic fugicides.

Key words: Plant extract · *Cerbera odollam* L. · Penicillium *digitatum* · Post-harvest disease · Orange

INTRODUCTION

Penicillium digitatum the cause of citrus green mold respect is important postharvest pathogens and cause serious losses annually [1]. The disease is currently managed with synthetic fungicides. However, these chemicals become pose a significant risk is widely, with continued use of fungicides chemicals on food agriculture crops due of their potential effects on human and the environment [2]. Pathogen resistance is another factor militating against the continuous use of synthetic fungicides [3]. The extensive use of agrochemicals especially fungicides, with more [4] carcinogenic risk than other pesticides [5] may give rise to undesirable effects on animals and human.

This damage increased significantly with the improper use and randomly led to then grow to reduce the use of these chemicals that accumulate in fruits, vegetables. Plant's extracts are one of several non-chemical control options that have recently received attention. However, actual use of these extracts to control post-harvest pathogens of fruits and citrus pathogens in particular is still limited [6].

Plan extracts from plant species *Withania somnifera* and *Acacia seyal* led to the inhibition of the growth of fungus *Penicillium digitatum* by up (70%) when used for 21 days under the conditions of storage of natural citrus [7]. Glucosinolates from mustard and horseradish also showed antimicrobial activity against *P. digitatum* [8]. Showed study [9] *in vitro* for evaluate (fenugreek seeds, harmal seeds, garlic cloves, cinnamon bark, sticky fleabane leaves and nightshade leaves and fruits) against *P. digitatum* that crude extracts of nightshade fruits cinnamon bark have completely inhibited the growth of tested fungal isolates and reached values LC50 to = 57.5 µg ml.

The plant extracts reported effective against the fungi *Penicillium digitatum* include *Allium sativum*, *Azadirachta indica*, *Withania somnifera* and *Acacia seyal* [10, 11]. The objective of this study is to evaluate

Corresponding Author: Ghassan F. Al-Samarai, School of Bioprocess Engineering, University Malaysia Perlis, Malaysia. Mob: +60174491361.

using of botanical pesticides as means to protect crops and their products and the environment from fungicides. Antifungal activity of crude extracts of five plants against green mould rot of citrus caused by *P. digitatum* under conditions *vitro* and *vivo* during storage.

MATERIALS AND METHODS

Pant Materials

Preparation of Plant Extracts: Plants were collected from a kitchen garden housing and local market in Kangar/Perlis and washed under running water, to get rid of dirt, insects and plankton. They were dried overnight in the laboratory- electric oven at 40°C. One hundred grams of the material (leaves, fruits) were pulverized by an electric mixer and preserved in labelled glass bottles that were sealed until use. The extraction technique used was a modification of [12] method. Fifty grams each of the oven dried and pulverized powered material from plants were treated with 500 ml of 95% alcohol with constant stirring for 30 minutes. After stirring, the solutions were filtered through 2 layers of cheese- cloth gauze and Whitman's (No.2) filter paper before subjecting the filtrates to evaporation in Rotary Evaporator at 60°C degree for 60 min. The dark spongy materials from the Rotary evaporator were removed and dried in an oven at 37°C for 2 days. The dried powder from the oven was stored in small, sterilized 5 ml screw-capped glass bottles and kept in the refrigerator (4°C) until further usage.

Preparations of Plant Extract Dilutions: Powder extracts from plants were removed from the refrigerator and were brought to the lab for the preparation of extract dilutions. Aliquots of 0.5,1.0 2.0, 3.0, 4.0 and 5.0g from each powder (plants) were mixed with organic solvent dim ethyl sulfoxide (DMSO) to obtain the concentrations required after the complete volume with distilled water to make dilutions of 500,1000, 2000, 3000 , 4000 and 5000ppm.

Pathogen: Using taxonomic and morphological references the pathogen identified was *Penicillium digitatum*. Highly aggressive, single-spore isolates of *P. digitatum*, originally isolated from citrus fruits were grown on potato dextrose agar (PDA) at 25°C for 7 days. Spores were harvested by flooding the media surface with sterile distilled water and gently agitating the plate to dislodge spores [6, 11]. Concentration spores were determined by use a hemacytometer and adjusted to $1x10^6$ spore's mL^{-1}.
In vitro screening.

Effect Plant Extracts in Mycelium Growth Penicillium Digitatum: PDA media was incorporated into forty-five in glass flasks (50 ml) and autoclaved for 20 min at 121°C. After autoclaving the flasks were cooled down to about 45°C. Five ml of each plant extract, (500, 1000, 2000 and 3000 ppm) aided using pipette in flasks that were gently agitated by hand for 2 min to allow for a proper mixing of extract. Media cultures were amended into 9cm in Petri-dishes. Chloramphenicol (250 ml/g per Petri dish) was added to the medium to prevent bacterial growth [12]. The experiment was performed under aseptic lamina conditions and replicated thrice. One ml, of *P. digitatum*, spore suspensions (conc.1×10^6 spores/ml^{-1}) were pipette on to the centre of the amended PDA extracts. Inoculated plates were incubated at 25o C for 10 days. The Petri-dish inoculated without the extract concentrations, served as control. Colony diameter was determined by measuring the average radial growth. The inhibition zone was measured using the formula of [13] as follows:

$$\% \text{ Mycelial inhibition} = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (control)}} \text{X } 100$$

***In vivo* Effect Plant Extracts on Postharvest Quality Parameters:** Healthy freshly citrus fruit washed with tap water and then air dried and sterilized by immersion in 70% ethanol for 1 min before spray. Fruits randomly divided into 5 equal groups (5 fruits); all groups were wounded in depth of 5.0 mm with a 1.25 mm diameter needle at the equator all treatments were inoculated by spray in suspension of *P. digitatum* ($1x10^6$ spore's mL^{-1}). Five treatments were carried out as following after one hour.

- Spraying with 2000ppm
- Spraying with 3000ppm
- Spraying with 4000ppm
- Spraying with 5000ppm
- Healthy Control (spraying with tap water)

Treated fruits were packed in plastic boxes and incubated at 25°C with >85% RH for 21 days. Evaluation the treatment was done every week. Efficacy of treatment application was determined according to [14]. Percentage of disease severity was calculated by dividing the weight of infected area by weight of orange. There were three replicate trials of 5 fruits per replicate. Representative samples of five fruits per replicate were taken each weekly storage period until the percentage of decay reached 50%.

Studies Character *In vivo*

Weight Loss%: This character was determined by weighing 5 fruits in each replicate after three week. Percentage of weight loss was calculated by determination of progressive reduction in fruit weight during storage period relative to the original fresh weight at the beginning of storage.

Undesirable Fruits Percentage: Calculated by dividing the number of decayed fruits by the total number of fruits as following equation:

Undesirable fruits% = (The number of undesirable fruits/Total number of fruit) x 100

Cytotoxicity Screening from Crude Fraction (LC50): Test Brine shrimp lethality is widely used in the bioassay for the in *in vivo*.

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RESULTS

Effect Plant Extracts in Mycelium Growth Penicillium Digitatum *In vitro*: The study showed different significant results (P>0.05, Fig. 1) when compared with the control in vitro. Results presented in Table 2 showed crude plant extracts that have been tested at concentration 1000,2000 and 3000 ppm (v/w) of Neem, Pong-Pong, hot Chili , Lemon grass and Ginger Inhibition of growth mycelia *P. digitatum*. The concentration at 3000ppm from Neem, Pong-pong and chilly showed significant decrease growth of *P. digitatum* with other extracts.

In vivo

Weight Loss%: Result showed that fruit weigh loss percentage increased by extending in storage period. These results could be due to the loss in moisture content. The results indicated that all treatments significantly decreased fruit weight loss percentage during the period of storage for 8 weeks, compare with controls. Experiments tested plant extracts spraying with Neem, Pong-pong and hot Chili showed significantly reduced fruit weight loss percentage by 21.3, 18.98 and 19.8%. Lemon grass and Ginger also recorded low fruit weigh loss percentage reached to 38.8 and 39.6 % respectively (Fig. 1).

Undesirable Fruits Percentage%: Results indicated that all treatments significantly reduced the undesirable fruits percentage during storage for 8 weeks. The best treatments were spraying with plant extract from Neem, Pong-pong and hot Chili at conc: 4000 and 5000ppm significantly reduced undesirable fruits percentage by 53.58, 52.50, 55.6, 53.16 and 52.90, 55.5% respectively compare with controls, Also showed the treatments spraying with Lemon grass and Ginger at 5000ppm some effect through reduce the undesirable fruits by 42.9 and 43.6%, Sequentially (Fig. 2).

Brine Shrimp Lethality Bioassay: The cytotoxic activities of all the extracts (n- Hexane, Ethyl acetate, Methanol solvents and water) of *Azadirachta indica* L., *Cerbera odollam* L., *Capsicum frutescence* L., *Zingiber officinale* L. and *Cymbopogon nardus* L. were studied by brine shrimp lethality bioassay The LC_{50} values of extracts of parts plant were 20(32-6), 5(15-0), 30(37-9), 473 (1818 - 212) 495 (858 – 306)µg mL^{-1}, respectively (Table 4).

Table 1: Plant species, parts and concentration used in experiment

Common name	Plan species	Family name	Nature of the extract	Conc: *in vitro* (ppm) (Petri dish)	Conc:*in vivo* (ppm) (store)
*Neem	*Azadirachta indica* L.	Meliaceae	Leaf powder	500, 1000.2000, 3000	2000.3000, 4000,5000
*hot Chili	*Capsicum frutescence* L.	Solanaceae	Fruit powder	500, 1000.2000, 3000	2000.3000, 4000,5000
*Pong-Pong	*Cerbera odollam* L.	Apocynaceae	Leaf powder	500, 1000.2000, 3000	2000.3000, 4000,5000
*Lemon grass	*Cymbopogon nardus* L.	Poaceae	Leaf powder	500, 1000.2000, 3000	2000.3000, 4000,5000
Tumeric	*Curcuma longa* L	Zingiberaceae	Tuber powder	500, 1000.2000, 3000	2000.3000, 4000,5000
Clove	*Syzygyum aromaticum* L.	Myrtaceae	Fruit powder	500, 1000.2000, 3000	-
Green chirayta	*Andrographis paniculata* L.	Andrographis	Leaf powder	500, 1000.2000, 3000	-
Mahogani	*Swietenia macrophyllai* L.	Meliaceae	Leaf powder	500, 1000.2000, 3000	-
Curry leaf	*Murraya koenigii* L	Rutaceae	Leaf powder	500, 1000.2000, 3000	-
*Ginger	*Zingiber officinale* L	Zingiberaceae	Tuber powder	500, 1000.2000, 3000	-

* Best plants that showed highest inhibition growth.

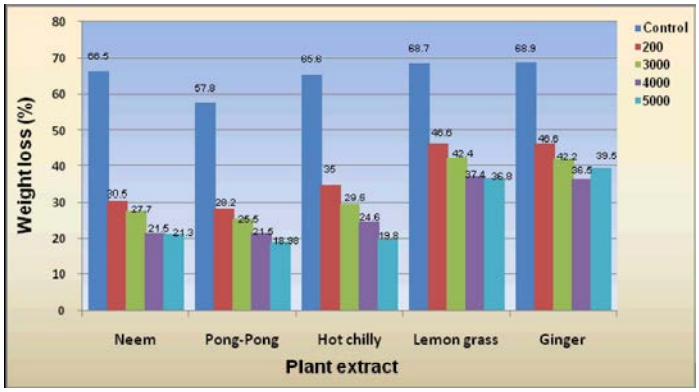

Fig. 1: Effect spray plant extracts at different concentrations of weight loss (%) in orange fruits after 8 weeks for storage at 25°C

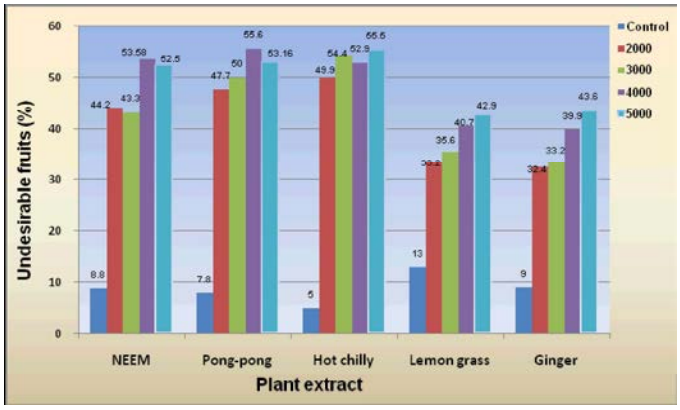

Fig. 2: Effect sprays plant extract at different concentrations of undesirable fruits (%) after 8 weeks for storage at 25°C

Table 2: Percentage of *P. digitatum* growth inhibition (cm^2) caused by plant extracts(ppm)in petri dishes PDA inoculated and incubated at 25o C for 10 days *in vitro*

	Treatment (ppm)				
	Control	500	1000	2000	3000
Plants	Colony diameter(cm^2)				
*Neem	8.133	4.26	2.73	1.50	0.00
*Hot Chilly	9.033	3.93	2.96	2.10	0.00
*Pong-Pong	9	2.76	1.99	1.46	0.63
*Lemon grass	8.66	3.90	2.96	2.33	1.03
Tumeric	8.90	4.20	3.33	2.73	1.73
Clove	8.13	5.96	5.74	5.13	4.76
Green chirayta	8.88	6.2	5.7	5.1	4.8
Mahogani	9.00	4.40	4.06	3.20	2.90
Curry leaf	8.5	6	5.8	5.5	5.2
*Ginger	8.46	4.10	2.60	2.20	1.43

Table 3: Effect of spraying plant extracts different concentrations of store on developing *P. digitatum* (%) of orange fruits (5 fruits each treatment) at 25°C

	Treatment (ppm)				
	Control	2000	3000	4000	5000
Plants	*Disease index (%)				
Neem	4.7	1	0	0	0
Hot Chilly	4	0.66	0	0	0
Pong-Pong	4	0.33	0	0	0
Lemon grass	4.33	3	3	3	3.3
ginger	5	3.6	3	3	2.7

*Disease index (decay reached 50 %.)

Table 4: Brine Shrimp test toxicity of plant extracts under study

Plants	Part used	Traditional use	LC50 ($\mu g/ml^{-1}$)
Azadirachta indica L (Neem)	Leafs	Anti-fungi	20(32-6)
Capsicum frutescence L(Pong-pong)	Leafs	Anti-fungi	5(15-0)
Capsicum frutescence L. (hot Chilly)	Fruits	Anti-fungi	30(37-9)
Zingiber officinale L.(Lemon grass)	Leafs	Anti-fungi	473 (1818 - 212)
Cymbopogon nardus L.(Ginger)	Tubers	Anti-fungi	495 (858 – 306)
Post control	Fungicide (quazatine)	Anti-fungi	326(540 – 198)
Negative control	DMSO	----------	>1000

DISSECTION

The resulted of the current research was to study the effect of plant extracts on the mycelium growth of, *Penicillium digitatum* that is pathogens for the post- harvest diseases of citrus as reported by [10]. These diseases could cause a loss of up to 10-30% decrease in crop yield and marketing quality [16, 17]. The use of biocontrol agents in plant disease control with plant extracts like lemon, citronella, clove, mint, thyme and oregano oils has been employed by [18] as alternative control measures to replace the conventional synthetic pesticides. The plant extracts reported effective against the fungi *Penicillium digitatum* include garlic, *Withania somnifera* and *Acacia seyal* and *mustard horseradish* [19, 11].

The reason of undesirable fruits percentage decay was the growth of mould green. These finding are in agreement with [20]. The activities of the plant extracts may be due to the action of their bioactive compounds against fungi growth through prevent growth spores such as *Penicillium italicum* and *P. digitatum*. These results are in agreement with [21]. They reported that the fungicidal of oil obtained from thymus against several post harvest pathogens may reveals the marked fungicidal activity of carvacrol in thyme. Found that the concentration of 900 ppm thyme essential oil prevented citrus fruit mould. Moreover, [22] reported that lime fruit peel essential oil components inhibit linear growth on spore germination of *P. italicum*, *P. digitatum* and *Geotrichum canium* [23]. In study by [14] evaluation effect of lime, thyme, comphore oils for their inhibitory effect *in vitro* different concentration of each essential oil at 1,5 and 10% (v/v) was tested on the growth of *P. digitatum*. The best concentration at 10% showed the highest inhibition growth of *P. digitatum* for all tested oils and significantly reduced the disease severity of fruits compared with untreated fruits (control) at 5°C.

Fruit weight loss% could be due to the loss in moisture content. This agrees with the finding of Elshiekh and [24]. The positive effects of oils on decreasing fruit weigh loss might be attributed to make a thin film of oil surrounding the fruit peel and induced a modification of microclimatic of fruits. These results are in agreement with [4, 23]. Also this result come similar with what reached by [14] significantly reduced the undesirable fruits percentage, fruit weight loss percentage during cooling storage for 14 weeks compared with control of each essential oil at 1, 5 and 10% (v/v) of lime, thyme, comphore oils.

This study is a general agreement with the results of earlier investigations [25, 26]. Some chemical compounds have been isolated from seed [7] and bark [27] of *S. mahogany*, in spite, this result should be encouraging other researchers to more study including photochemical and biological investigation. The earlier reports of antimicrobial activities [28] support the findings of present studies. Inhibition effects of crude extracts indicate that it can be selected for more of the application, because there is a correlation between effectiveness of the extracts and activity against the brine shrimp nauplii using extracts.

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