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# Exploitation of Natural Products as an Alternative Strategy to Control Postharvest Fungal Rotting of Citrus

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***Abstract-*** Two alcoholic extracts from *Capsicum frutescence* L. (Chilly) and *Zingier officinale* L. (Ginger) (ranging between 500 and 3000 ppm) were tested for antifungal activity *in vitro* on *Penicillium digitatum*, *Aspergillus nige*r and *Fusarium sp* isolated from naturally infected citrus fruit. The water extracts served as control and it was observed that the alcoholic extracts concentrations were more effective than the water extract control in showing antifungal activity ($P<0.05$) against the test pathogens. Results show the alcoholic extract concentrations were more effective than the water extract control in showing antifungal activity ($P<0.05$) against test pathogens. All 3000ppm concentration from *Capsicum frutescence* L. and *Zingier officinale* L. showed a 100% and 85% inhibition zone for all the three fungi respectively. Work is currently focusing on the mechanisms underlying the impacts of plant extracts on disease development with a major contribution to limiting the spread fungi to control post-harvest diseases in fruits.

***Index Terms***- natural products, *Capsicum frutescence* L., **post**-harvest pathogens, disease management

## I. INTRODUCTION

Postharvest diseases, such as soft rot of fruits, due to fungal infections cause significant economic losses for the citrus industry during storage, transport and marketin. The predominant pathogens causing the most important postharvest disease of fruits worldwide according to Poppe *et al* ;( 2001), are *Penicillium digitatum, Aspergillus niger* and *Fusarium sp.*, respectively.

Traditionally, plant disease control is achieved mainly through the use of fungicides such as Imazalil, Guazatine and Prochloraz. The use of fungicides is becoming more restricted due to health concerns (Ragsdale *et al*; 1994). It is therefore necessary to develop alternatives to synthetic chemical control to reduce environmental risks and raise consumer confidence. In this respect, derivatives from plant agents tend to show a potential alternative to synthetic fungicides (Zhang *et al*; 2005).

Environmentally friendly plant extract agents have shown great potential as alternatives to synthetic fungicides (Janisiewicz and Korsten, 2002; Zhang *et al*., 2005). Recently, the antimicrobial activity of biodegradable and safe higher plant products Kumar *et al* (2008) has attracted the attention of microbiologists. However, the actual use of these products to control postharvest pathogens of fruits, particularly citrus pathogens, is still limited. The purpose of the current research is to test the possibility of using extracts from chilly and ginger to control or inhibit post-harvest diseases causing pathogens in citrus fruits.

## II. METHODS AND METHOD

### *A. Collection of diseased fruits*

Wet markets at Kangar (Perlis) and Georgetown (Penang) were surveyed in December 2010, to observe common post-harvest disease symptoms in oranges, lemons, and grape fruits. The prominent symptoms observed were the growth of green, black, white colored molds on the fruits. Random samples were collected from citrus fruits and brought to the Microbiology laboratory of the School of Bioprocess Engineering, University Malaysia Perlis for further studies. The fruits were washed with water, disinfected with 10 % sodium hypochlorite, and cultured in sterilized PDA media under aseptic lamina conditions, for identification, single-spore isolation, and propagation under laboratory conditions at 25$^{o}$C.

### *B. Pathogens*

The pathogens identified using the taxonomic and morphological references were *Aspergillus niger, Penicillium digitatum,* and *Fusarium sp.* Highly aggressive, single-spore isolates of *P. digitatum, A.niger and Fusarium sp.* originally isolate from citrus fruits were grown on potato dextrose agar (PDA) at 25$^{o}$C for 7 days. The spores were harvested by flooding the media surface with distilled water and gently agitating the plate to dislodge spores (Obagwu and Korsten, 2002). The spores were then refrigerated for further studies.

### *C. Plants for extractions*

Chilly (fruits) were collected from a kitchen garden housing-estate Kangar. Ginger (rhizomes), collected from the local wet market of Kangar. The collected samples were washed under running water, to get rid of dirt, insects and plankton. Subsequent they were dried overnight in the laboratory-electric oven at 40$^{O}$C. One 100g of the material (fruits and rhizomes) was pulverized using an electric mixer, and preserved in labeled glass which were sealed until use.

### *D. Preparation of plant extracts*

The extraction technique used was a modification of Ruch's (2001) method. Up to 50g each of the oven dried and pulverized powered material from chilly and ginger were treated with 500 ml of 95% alcohol with constant stirring for 30 min. After stirring, the solutions were filtered through 2 layers of cheese-cloth gauze and Whitman's (No.2) filter paper before the filtrates were subjected to evaporation through Rotary Evaporator at 60$^{o}$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 was stored in small and sterilized 5ml screw-capped glass bottles they were refrigerator (4°C) until further use.

*E. Preparations of plant extract dilutions*

The chilly a ginger powder extracts were removed from the refrigerator and were brought to the lab for the preparation of extract dilutions. Aliquots of 0.5 g, 1.0g, 2.0g and 3.0g 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, and 3000 ppm.

## III. IN VITRO SCREENING

PDA media was incorporated in forty-five 50 ml glass flasks and autoclaved for 20 min. After autoclaving, the flasks were cooled to about 45°C. Approximately 5ml of plant extract,(500, 1000, 2000, and 3000 ppm) were taken form the Suicide tree, Clove, and Mahogany. They extract were pipette into four of the forty-five 50 ml flasks and were gently agitated by hand for 2 min for a proper mixing of extract. Up to 20 ml aliquots of the mixed media were dispensed into 9cm petri-dishes. Subsequently Chloramphenicol (250 mL/g per petri dish) was added to the medium to prevent bacterial growth (Nikos *et al.*, 2007). The experiment was performed under aseptic lamina conditions and replicated thrice. Approximately 1mLfrom *P.digitatum*, *A.niger* and *Fusarium.sp* (conc.$1\times10^6$ spores/mL) were pipette on the center of the amended PDA extracts. The inoculated plates were then incubated at 25°C for 10 days. The petri-dishes inoculated without the extract concentrations served as control. Moreover colony diameter was determined by measuring the average radial growth. The inhibition zone (P), was measured using the formula of Francisco (2010):

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$$P = \frac{(C - T)}{C} \times 100$$

Where C is the colony $cm^2$ of the control and T is of the treatments (three replicates).

## IV. STATISTICAL ANALYSIS

The experimental data was subjected to analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan's Multiple Range test ($P< 0.05$) following ANOVA. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, USA).

## V. RESULT

Culture PDA media with chilly and ginger extracts - enrichment resulted in significant ($P<0.05$) reduction on subsequent colony development *P. digitatum.* , *A.niger* and *Fusarium. Sp.* Mixing culture PDA media with all concentration, 0 (control) , 500, 1000, 2000, and 3000ppm of the plant extracts of the *Zingier officinale* showed significant results ($P<0.05$,Fig.1) when compared with the control . *Penicillium digitatum* showed a reduction in colony development ranging from an average of 51.5% .69.2%, 74. %, and 83.1% at concentration of 500, 1000, 2000, and 3000ppm respectively.

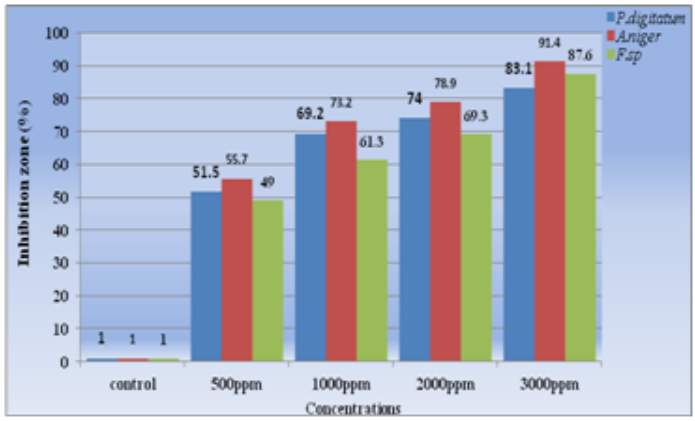


Fig. 1 Impacts of ethanolic extract of *Zingier officinale* L. (Ginger) expressed as % of inhibition zone on colony growth ($cm^2$) of *Penicillium digitatum, Aspergillus niger* and *Fusarium sp* raised on PDA and incubated at 25°C.

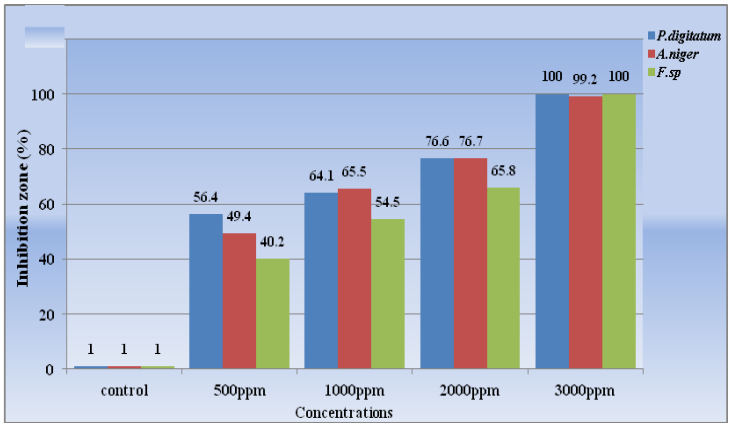


Fig. 2 Impacts of ethanolic extract of *Capsicum frutescence* L. (Chilly) expressed as % of inhibition zone on colony growth ($cm^2$) of *Penicillium digitatum, Aspergillus niger* and *Fusarium sp* raised

*Aspergillus niger* recorded inhibition zone of 55.7%.73.2%, 78.9% and 91.4% at similar plant extract concentration respectively. The inhibition zone observed in *Fusarium sp* were 49%,61.3%,69.3% and 87.6% respectively at concentration in the ascending order. From (Figure1), it is also observed that the 3000 ppm showed the best result in inhibiting the mycelial growth in all the three fungi studied .

Result on the efficacy of chilly extract on the post-harvest in citrus is presented in Figure2. A similar trend as the zinger extract was observed in its microbial inhibition activity ($P<0.05$), except that at 3000ppm, all the 3 fungi, namely *Penicillium digitatum, Aspergillus niger* , and *Fusarium sp*. recorded almost 100% inhibition zone.

The impacts of different zinger and chilly concentrations on the inhibition diameters of the fungi are presented in Table 1. From the data, it is observed that, the concentration of 3000 ppm gave the best inhibition zones with both the extracts.

Table 1: Impacts of extracts of *Capsicum frutescence* L.(Chilly) and *Zingier officinale* L. (Ginger) plant extracts on colony growth ($cm^2$) of *Penicillium digitatum* , *Aspergillus niger* and *Fusarium sp* raised on PDA.

| Tret(ppm) | *Capsicum frutescence* L. | | | *Zingier officinale* L. | | |
|---|---|---|---|---|---|---|
| | *P.digitatum* *CD (cm2 | *A.niger* CD (cm2 ) | *F. sp* CD($cm_2$) | *P. digitatum* CD($cm_2$) | *A.niger* CD($cm_2$) | *F. sp* CD($cm_2$) |
| Control | 9.033 ±0.033 | 9.033 ±0.179 | 7.033 ±0.177 | 8.467 ±0.120 | 9.333 ±0.088 | 6.733 ±0.176 |
| 500 | 3.933 ±0.328 | 4.567 ±0.189 | 4.200 ±0.153 | 4.100 ±0.115 | 4.133 ±0.115 | 3.433 ±0.100 |
| 1000 | 2.969 ±0.285 | 3.100 ±0.100 | 3.200 ±0.100 | 2.600 ±0.115 | 2.500 ±0.066 | 2.600 ±0.066 |
| 2000 | 2.100 ±0.285 | 2.100 ±0.115 | 2.400 ±0.057 | 2.200 ±0.057 | 1.966 ±0.088 | 2.066 ±0.484 |
| 3000 | 0.00 ±0.00 | 0.033 ±0.333 | 0.00 ±0.00 | 1.430 ±0.057 | 0.800 ±0.176 | 0.833 ±0.888 |

*CD refers to colony diameter

## VI. DISCUSSION

The plant extracts of chilly and ginger of this study was to evaluate the efficacy of botanicals in controlling three fungal pathogen (mycelia growth)of , *Penicillium digitatum*, *Aspergillus niger* and *Fusarium sp* that are pathogens for the post-harvest diseases of citrus as reported by Eckert & Sommer, (1967), and Adaskaveg *et al*, (2002). These diseases could cause a loss of up to 10-30% decrease in crop yield and marketing quality (Agrios, 2005, and Serrano *et al*, 2005).

In vitro studies of oregano, thyme, lemongrass, and cilantro vapours (500–1000 ppm) showed complete growth inhibition of B. *cinerea* and *Alternaria arborescence*. Geotrichum candidum was more sensitive to lemongrass oil vapours than to thyme or oregano oils (Plotto *et al*., 2003).

The plant extracts reported effective against the fungi *Penicillium digitatum* include garlic (Obagwa, 2002), neem (Mossini,.*et al*,2009), *Withania somnifera* L. and *Acacia seyal* L. Samson, 1984), mustard and horseradish (McOnie,1964).

*Aspergillus niger* is noted for its carcinogenic aflatoxin production in diseased plants. Montes and Carvjal (1998) in their research for screening of more than 280 plant species for their inhibitory effect on the toxin reported that about 100 of these plants had some activity on growth of toxin production by fungi. Clove completely inhibited the mycelia growth of *A. flavus* and aflatoxin formation (Karapynar, 1989).

Saxena and Mathela (1996) in their study on the inhibitory effect of plant extracts on *Fusarium* reported that, *Azadirachta indicia* L., Artemisia *annua* L., *Eucalyptus globules* L., *Ocimum. Sanctum* L. and *Rheum emodi* L., showed significant reduction of the pathogen. Garlic extract had a positive effect on the *Fusarium* inhibition (Anjorin.*et al*, 2008).

## VII. CONCLUSION

Most plant derivatives, phenols and alkaloids tend to show positive effect on the inhibition of postharvest fungal or bacterial pathogens. Amidst an increasing global environmental pollution, these plant extracts or botanicals have great replace potential replacing conventional synthetic pesticides in the future.

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