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Performance of botanical pesticides to control post-harvest fungi in citrus

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Abstract— Three alcoholic extracts from *Cerbera odollam* L.(Suicide tree) , *Syzygium aromaticum* L. (Clove) and *Swietenia macrophyllai* L. (Mahogany) at concentrations of 500 ppm, 1000 ppm, 2000ppm and 3000ppm, were tested for antifungal activity *in vitro* on *Penicillium digitatum, Aspergillus niger* and *Fusarium sp* isolated from naturally infected citrus fruit. The water extracts served as control. 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 *Cerbera odollam* L. showed a 90% inhibition zone for all the three fungi. The inhibition zone of *Syzygium aromaticum* L .and *Swietenia macrophyllai* L. were 40% and 60% respectively, at the same 3000 ppm concentration. Plant extracts are viable alternatives to chemical pesticides; they are readily available non-pollutive, cost effective, non-hazardous, and they do not disturb ecological balance. Moreover, Investigation are to test the efficacy of these extracts practical application.

Index Terms— Plant extracts, *Cerbera odollam* L., Post-harvest pathogens, Disease management, Botanical biopesticide

1 Introduction

Post-harvest diseases account for 50% of losses in fruits stored under poor storage conditions especially under high humidity. They pose a major problem to the agriculture industry (Agrios, 2005). Citrus fruits are one of the crops susceptible to post-harvest diseases caused by fungi under poor storage conditions. The most important fungi causing post-harvest diseases include: *Penicillium spp, Aspergillus niger, Monilinia lax,* and *Rhizopus stolonifer* (Ogawa *et al*., 1995) . Many fruits are prone to damage caused by insects, animals, early splits, and mechanical harvesting. The damage predispose the fruit to wound invading pathogen *Aspergillus flavus*, and other fungi, that causes the decay to spread in stored citrus fruits. *Aspergillus flavus* can pose as a serious health problem because of: its production of aflatoxin, which is a group of toxic and carcinogenic compounds (Diener *et al*., 1987*;* Wilson and Payne 1994 *;* Palumbo *et al*., 2006).

Synthetic fungicides, such as, thiabendazole, imazalil and sodium ortho-phenyl phonate (Poppe *et al*., 2003) have been used traditionally to control postharvest diseases. However, their excessive use, high cost, residues in plants, and development of resistance, have left a negative effects on human health and the environment (Paster and Bullerman, 1988; Bull *et al*., 1997). 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 Suicide, Clove, and Mahogany trees to control or inhibit post-harvest diseases causing pathogens in citrus fruits.

2. Materials and Method

2.1 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, or blue 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°C.

2.2 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 isolated from citrus fruits were grown on potato dextrose agar (PDA) at 25°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 and propagation.

2.3 Preparation of plants for extraction

Cerbera odollam L (Suicide tree), Syzygium aromaticum L. (Clove), and Swietenia macrophyllai (Mahogany) were col-

lected from a kitchen garden housing-estate Kangar. The collected samples were washed under running water, to get rid of dirt, insects and plankton. Subsequently they were dried overnight in the laboratory-electric oven at 40c. One 100g of the material (leaves and fruits) were pulverized using an electric mixer, and preserved in labelled glass which were sealed until use.

2.4 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 *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemon grass) *Zingiber officinale* (Ginger), *Capsicum frutescent* (Chilly) *Syzygium aromaticum* (clove) 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°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.

2.5 Preparations of plant extract dilutions

The Suicide tree, Clove and Mahogany powder extracts were removed from the refrigerator and were brought to the lab for the preparation of extract dilutions. Aliquots of 1.0g, 2.0g an3.0g 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 ppm, 1000 ppm, 2000 ppm, and 3000 ppm.

2.6 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 ppm, 1000 ppm, 2000 ppm, and 3000 ppm) were taken form the Suicide tree, Clove, and Mahogany. They extracts were pipetted 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 1ml from *P. digitatum, A.niger* and *Fusarium. Sp* spore suspensions (conc.1 × 10^6 spores/ml) were pipetted on the center of the amended PDA extracts. The inoculated plate was 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):

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

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

3. 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).

4. Result

The post-harvest fungi which were identified on the basis of their cultural and morphological characteristics, and tested for anti microbial activity were *Penicillium digitatum, Aspergillus niger,* and *Fusarium sp*. Mixing culture PDA media with all concentrations (0 ppm (control), 500 ppm, 1000 ppm, 2000 ppm, and 3000 ppm) of the plant extracts from *Cerbera odollam* L. showed significant results (P>0.05, Fig. 1) compared with the control. *Penicillium digitatum* showed a reduction in colony development in ascending order; ranging from 69.3%, 77.8 %, 83.7 %, and 93 % at concentrations of 500, 1000, 2000, and 3000 ppm respectively. *Aspergillus niger* recorded inhibition zones of 74%, 80.7%, 86.6%, and 95.1% at similar plant extract concentrations. The inhibition zones observed in *Fusarium sp* were 57.7% 68.5%, 73.1% and 95%. No inhibition zone was seen in control treatments. The results show that 3000 ppm achieved the best results in inhibiting mycelial growth among three fungi the three fungi studied.

Result of the efficacy of plant extracts on post-harvest pathogens in citrus fruits are presented in Figures.1, 2, and 3. A different trend in the microbial inhibition activity (P>0.05) of the *Cerbera odollam L.* extract was observed with all three fungi (Aspergillus *niger, Penicillium digitatum*, and Fusarium *sp*) except at 3000ppm.

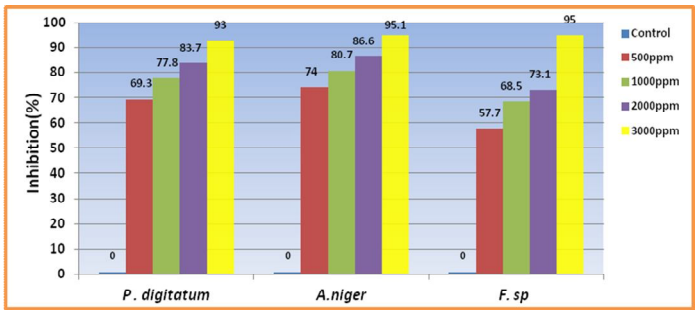

Figure1. Impacts of ethanolic extract of *Cerbera odollam* L .(Suicide tree) expressed as % of inhibition zone on colony growth (cm^2) of *Pencillium digitatum, Aspergillus niger* and *Fusarium sp,* raised on PDA and incubated at 25°C.

Tret (ppm)	C. odollam			S.aromaticum			S.macrophyllai		
	P.d	A.n	F.sp	P.d (CD)*	A.n	F.sp	P.d	A.n	F.sp
cont	9.00	9.36	6.55	8.13	9.26	6.66	9.00	9.46	6.56
500	2.76	2.70	2.77	5.96	5.90	5.16	4.40	4.66	4.33
1000	1.99	1.80	2.06	5.74	5.56	4.46	4.06	3.93	2.56
2000	1.46	1.26	1.76	5.13	5.13	4.46	3.20	3.56	3.26
3000	0.63	0.45	0.22	4.76	4.86	4.03	2.96	2.90	2.48

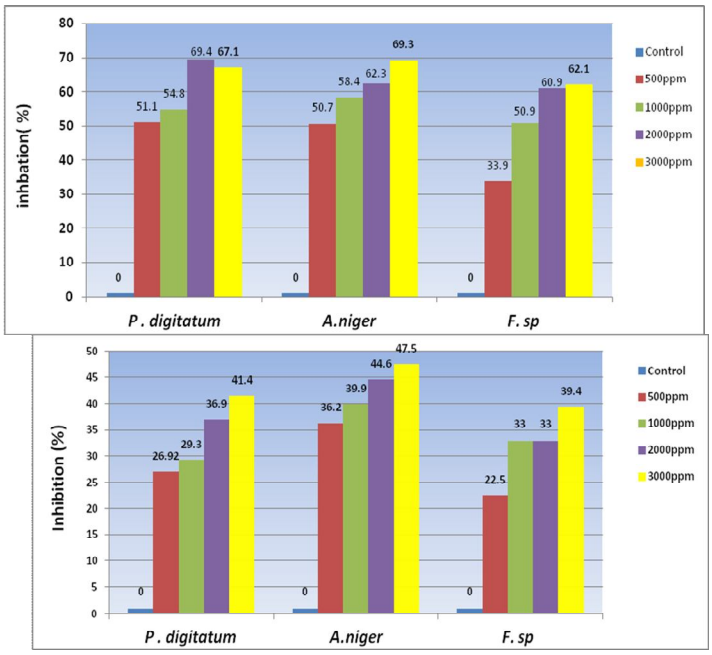

Figure 2. Impacts of ethanolic extract of *Syzygium aromaticum* L .(Clove) expressed as % of inhibition zone on colony growth (cm^2) of *Pencillium digitatum, Aspergillus niger* and *Fusarium sp* raised on PDA and incubated at 25°C.

Figure 3. Impacts of ethanolic extract of *Swietenia macrophyllai* L. (Mahogany). expressed as % of inhibition zone on colony growth (cm^2) of *Pencillium digitatum, Aspergillus niger* and *Fusarium sp* raised on PDA and incubated at 25°C.

To modify the running headings, select View Header and Footer. Click inside the text box to type the name of the journal the article is being submitted to and the manuscript identification number. Click the forward arrow in the pop-up tool bar to modify the header or footer on subsequent pages. The impact of concentrations on the inhibition diameters of the fungi are presented in Table 1. The data shows different effects. *Syzygium-aromaticum* L. gives slow inhibition zones (40%) for both extracts at concentrations of 500ppm to 3000 ppm, in contrast with the control treatment. The impact medial of *Swietenia macrophyllai* L. in the inhibition zone on colony growth (60%) is also given

Table 1. Impacts of extracts of *Cerbera odollam* L., *Syzygium aromaticum* L. and *Swietenia macrophyllai* plant extracts on Colony Diameter [CD] in cm of *Penicillium digitatum, Aspergillus niger* and *Fusarium sprose* on PDA

CD* refers to colony diameter *P.d= Penicillium digitatum*
A.n= Aspergillus niger *F.sp = Aspergillus niger*

5- Discussion

The objective of the current research is to study the effect of plant extracts on the mycelia growth of, *Penicillium digitatum, Aspergillus niger* and *Fusarium sp* that are pathogens of the post-harvest diseases of citrus fruits, as reported by Eckert & Sommer,(1967),and Adaskaveg *et al*, (2002). These diseases have caused 10-30% decrease in crop yield and marketing quality (Agrios, 2005, and Serrano *et al*., 2005).

The use of biocontrol agents from plant extracts like lemon, citronella, clove, mint, thyme and oregano oils has been employed by Viudamartos *et al* (2007), as alternatives for conventional synthetic pesticides in plant disease control. The plant extracts that are reportedly effective against the fungi *Penicillium digitatum* include garlic (Obagwa,2002), neem (Mossini, *et al*.,2009), *Withania somnifera* and *Acacia seyal* (Samson, 1984),and mustard and horseradish (McOnie,1964).

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

Garlic extract has a positive effect on *Fusarium* inhibition (Anjorin *et al*., 2008). Saxena and Mathela (1996), in their study on the inhibitory ;

effect of plant extracts on *Fusarium,* have reported that, *Azadirachta indicia, Artemisia annua, Eucalyptus globules, O.cimum, Sanctum and Rheum emod* , have shown significant reduction of pathogens. In the current research, the Suicide tree at 3000 ppm has been discovered to shows almost 95 % inhibition of the mycelia growth in culture medium.

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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REREFRANCES

[1] Adaskaveg, J.E., H. Forster, N.F. Sommer. (2002). Principles of post-harvest pathology and management of decays of edible horticultural crops. In: *Post-harvest Technology of Horticultural* Crops, (Eds.): A.Aader. Vol. 3311. Universi-

ty of California Publication, California, Pp. 163-195. Agrios, G.N., (2005). *Plant Pathology, Academic Press*, New York.

[2] Anjorin, S.T., H.A. Makun, T. Adesina and I. Kudu.,(2008). Effects of *Fusarium verticillioides*, its metabolites and neem leaf extract on germination and vigour indices of maize (Zea mays L.) .Afr. j.Biotechnol. 7:2402-2406.

[3] Bull, C.T, Stack, J.P, Smilanick, J.L.,(1997). Pseudomonas syringae strains ESC-10 and ESC-11 survive in wound on citrus and control green and blue molds of citrus. *Biol. Contr*. 8: 81-88.

[4] Diener, U.L, Cole, R.J, Sanders, T.H, Payne, G.A, Lee, L.S, Klich, M.A.,(1987) .Epidemiol –ogy of aflatoxin formation by Aspergillus flavus. *Ann. Rev. Phytopathology*. 25: 249-270.

[5] Eckert, J.W. and N.F. Sommer.(1967)., Control of diseases of fruits and vegetables by post-harvest treatment. Ann. Rev. *Plant Pathol*., 5: 391-432.

[6] Francisco, D,H., (2010). Lippia graveolens and Carya illinoensis Organic Extracts and there in vitro Effect against Rhizoctonia Solani Kuhn. *American Journal of Agricultural and Biological Sciences* 5(3): 380-384.

[7] Janisiewicz, W.J., Korsten, L.,(2002). Biocontrol of post harvest diseases of fruits. Annu. Rev. *Phytopathology*.40: 411-441.

[8] Obagwa, J., Korsten, L., (2002). Control of citrus and green and blue molds with garlic extracts. *Plant Pathology*.109, 221- 225.

[9] Karapynar.,(1989). Inhibition effects. Of some spice agents on aflatoxigenic mould growths. *Proceedings of the International Food Symposium, April 4-6, Bursa, Tukey, pp:129*-137.

[10] Kumar, A., Shukla, R., Singh, P., Prasad, C.S., Dubey, N.K., (2008). Assessment of Thymus vulgarism essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innu. *Food Sci. Emerg*., 4: 575-580.

[11] Ogawa, J.M., Dehr, E.I., Bird, G.W., Ritchie, D.F., Kiyoto, V., Uyemoto, J.K., (1995). *Compendium of Stone fruit Diseases*. APS Press, USA.

[12] McOnie, K. C., (1964). The latent occurrence in citrus and other hosts Guignardia easily confused with G. citricarpa, the black spot pathogen. *Phytopathology* 54:40- 43.

[13] Mossini, S.A.G., C. Carla and C. Kemmelmeier.,(2009). Effect of neem leaf extract and Neem oil on Penicillium growth, sporulation, morphology and ochratoxin A production. Toxins, 1: 3-13.

[14] Nikos, G.T and C. D. Economakis.,(2007).Antifungal activity of lemongrass (Cymbopogon citrates L.) essential oil against key postharvest pathogens. *Global Journal of Biotechnology & Biochemistry* 3 (2): 56-59, 2008.

[15] Palumbo, J.D., Baker, J.L., Mahoney, N.E., (2006). Isolation of bacterial antagonists of Aspergillus flavus almonds. *Microbial Ecol.* 52(1): 45- 52.

[16] Paster, N., & Bullerman, L. B., (1988). Mould spoilage and mycotoxin formation in grains as controlled by physical means. *International Journal of Food Microbiology*, 7, 257−265.

[17] Poppe, L., Vanhoutte, S., Höfte, M., (2003). Modes of action of Pantoea agglomerans CPA-2, an antagonist of postharvest pathogens on fruits. *Eur. J. Plant Pathol*. 109: 963-973.

[18] Ruch, B.a. Worf, R.(2001) . Processing of neem for plant protection simple and sophisticated standardized extracts. Abstracts of the .Work shop, Neem and Pheromones , University of Uberaba ,Brazil, March 29-30 Augusts, P.499.

[19] Samson, J.A., (1984).Tropical fruits- Tropical agricultural series. Longman Inc., New York, pp.64-118.

[20] Saxena. and C.S. Mathela. (1996). An activity of new compounds from Nepeta leak and Nepeta clarkei. *Applied Environ Microbiol*. 702- 704.

[21] Serrano, M., D. Martinez-Romero, S. Castillo. Guillen and D. Valero., (2005). The use of the natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food and Emerging Technologies*, 6: 115-123.

[22] Wilson, D.M., Payne, G.A.,(1994). Factors affecting Aspergillus flavus group infection and aflatoxin contamination of crops, In the Toxicology Aflatoxins. Human Health, Veterinary, and Agricultural Significance (Eaton DL, Groopman JD, San Diego: Academic Press).

[23] Viudamartos, M. Ruiz-navajas, Y., Fernandez -lopes, J. and Perez-alvarez, J.A.,(2007). Antifungal activities of thyme, clove and oregano essential oils. *Journal of Food Safety* 27: 91-101.

[24] Zhang, H., Zheng, X.,(2005). Biological control of postharvest blue mold of oranges by Cryptococcus laurentii (Kufferath) Skinner. Biocontrol 50:331-342.