2011 International Conference on Biomedical Engineering and Technology
IPCBEE vol.11 (2011) © (2011) IACSIT Press, Singapore

Anti-fungal activity of *Capsicum frutescence* and *Zingiber officinale* against key post-harvest pathogens in citrus

Harbant Singh, Ghassan Fairs and Mohd. Syarhabil

School of Bioprocess Engineering University Malaysia Perlis, E-mail contact: harbant@unimap.edu.my

Abstract. Alcoholic extracts of *Capsicum frutescence* (Chilly) and *Zingiber officinale* (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. The results also indicated that *Capsicum frutescence* showed 100% inhibition of colony diameter (cm^2) at 3000 ppm. Hence, the results of the present investigations indicate the plant extracts posses' antifungal activity that can be exploited as an ideal treatment for future plant disease management to eliminate fungal spread.

Key words: Plant extracts, *Capsicum frutescence*, *Zingiber officinale*, Post-harvest pathogens, Disease management

1. INTRODUCTION

Post-harvest diseases account to about 50% losses in fruits stored in poor storage conditions especially under high humidity. They are posing a major problem to the agriculture industry (Agrios, 2005). Citrus fruits are among 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* spp, *Alternaria* spp, *Botrytis cinerea*, *Monilinia lax* and *Rhizopus stolonifer* (Ogawa *et al*., 1995). Many fruits are prone to damage caused by insects, animals, early splits, and during mechanical harvesting. This damage predispose the fruits to the wound invading pathogen *Aspergillus flavus* ,and other fungi, that causes decay on stored citrus fruits. *Aspergillus flavus* can pose a health problem, especially it produces aflatoxin, a group of toxic, 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) has been used traditionally to control the postharvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment (Paster and Bullerman, 1988, Bull *et al*., 1997).

Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides (Janisiewicz and Korsten, 2002; Zhang *et a*l., 2005). Recently, the antimicrobial activity of some higher plant products that are biodegradable and safe to human health (Kumar *et al*., 2008) has attracted the attention of microbiologists in the control of plant disease, but the actual use of these products for the control of postharvest pathogens of fruits generally, and in particular for citrus pathogens is, however, still limited. The purpose of our research is to test the possibility of using extracts from chilly and ginger to control or inhibits the pathogens causing post-harvest diseases in citrus fruit and is presented in this paper.

2. MATERIALS AND METHODS

Collection of diseased fruits

Wet markets at Kangar (Perlis) and Georgetown (Penang) were surveyed in December 2010, to observe the common post-harvest disease symptoms in citrus fruits, namely, from orange, lemon, and grape fruit. The prominent symptoms observed were the growth of green, black, white or bluish - mold on the fruits. Random samples were collected from the citrus fruits and brought to the Microbiology laboratory at the School of Bioprocess Engineering, University Malaysia Perlis for further studies. They 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 the laboratory conditions at 25^{o}C.

Pathogens

Using taxonomic and morphological references the pathogens identified 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^{o}C for 7 days. Spores were harvested by flooding the media surface with sterile distilled water and gently agitating the plate to dislodge spores (Obagwu and Korsten, 2002) and kept in the refrigerator for further studies and propagation.

Preparation of chilly and ginger plants for extractions

Chilly plants (hot pungent local chilly paddy, *Capsicum frutescence*) were collected from a kitchen garden housing estate in Kangar and washed under running water, to get rid of dirt, insects and plankton. They were dried overnight in the laboratory- electric oven at 40^{0}C. One hundred grams of the material (stems, leaves, fruits) were pulverized by an electric mixer, and preserved in labelled glass bottles that were sealed until use.

The ginger (*Zingiber officinale)* rhizomes, collected from the local wet market of Kangar, were subjected to the same procedure.

Preparation of plant extracts

The extraction technique used was a modification of **Ruch's (2001**) method. Fifty grams each of the oven dried and pulverized powered material from *Zingiber officinale* (Ginger), *Capsicum frutescence* (Chilly), 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 Buchi 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^{o}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^{o}C) until further usage.

Preparations of plant extract dilutions

The chilly and zinger powder extracts were removed from the refrigerator and brought to the lab for the preparation of extract dilutions. Aliquots of 0.5 g, 1.0 g, 2.0 g and 3.0 g of each powder (chilly and ginger) were mixed with distilled water to make dilutions of 500 ppm, 1000 ppm, 2000 ppm, and 3000 ppm.

In vitro screening

PDA media was incorporated into **forty-five** 50 ml glass flasks and autoclaved for 20 min. After autoclaving the flasks were cooled down to about 45^{0}C. Five ml of each plant extract, (500 ppm, 1000 ppm, 2000 ppm, and 3000 ppm, from chilly and zinger) was pipetted into **4** of the above 50 ml flasks that were gently agitated by hand for 2 min to allow for a proper mixing of extract.

20 ml aliquots of the amended media were dispensed into **five** 9 cm Petri-dishes. 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.

One ml, each, of *P. digitatum. , A.niger* and *Fusarium. sp* spore suspensions (conc.1 × 10^{6} spores/ml) were pipetted on to the centre of the amended PDA extracts. Inoculated plates were incubated at 25^{o} 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 **(P)**, was measured using the formula of Francisco (2010) as follows:

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

Where **C** is the colony cm^2 of the control and **T** is of the treatments (3 replicates).

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)

3. RESULTS

The post-harvest fungi, identified on basis of their cultural and morphological characteristics and tested for the anti microbial activity of the plant extracts were, Aspergillus *niger, Penicillium digitatum,* 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 of the *Zingiber officinale* (Ginger) , 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 52%, 69%, 74%, and 83% at concentrations of 500, 1000, 2000, and 3000 ppm respectively. *Aspergillus niger* recorded inhibition zones of 56%, 73%, 78%, and 91% at similar plant extract concentrations respectively. The inhibition zones observed in *Fusarium* sp were 49%, 61%, 69% and 88% respectively at concentrations in the ascending order. The control treatments showed no inhibition zones. From Figure 1, it is also observed that the 3000 ppm showed the best result in inhibiting the mycelial growth in all the 3 fungi studied.

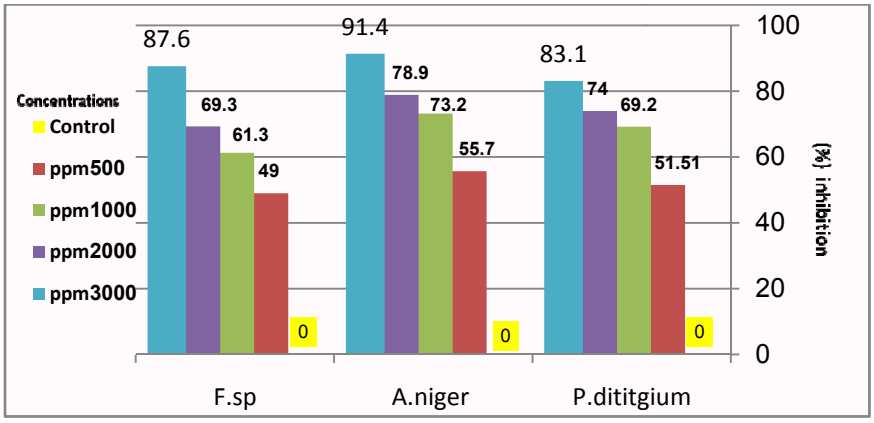

Figure 1Impacts of Ginger (*Zingiber officinale* L.) plant extract on colony growth of (cm^2) *Penicillium digitatum , Aspergillus niger and Fusarium sp* raised on PDA. Plates were incubated in controlled environment champers maintained at 25^0C .

Result on the efficacy of chilly extract on the post-harvest pathogens in citrus is presented in Figure 2. A similar trend as the zinger extract was observed in its microbial inhibition activity (*P>0.05)*, except that at 3000 ppm, all the 3 fungi, namely, *Aspergillus niger, Penicillium digitatum,* and *Fusarium* sp. recorded almost 100% inhibition zones.

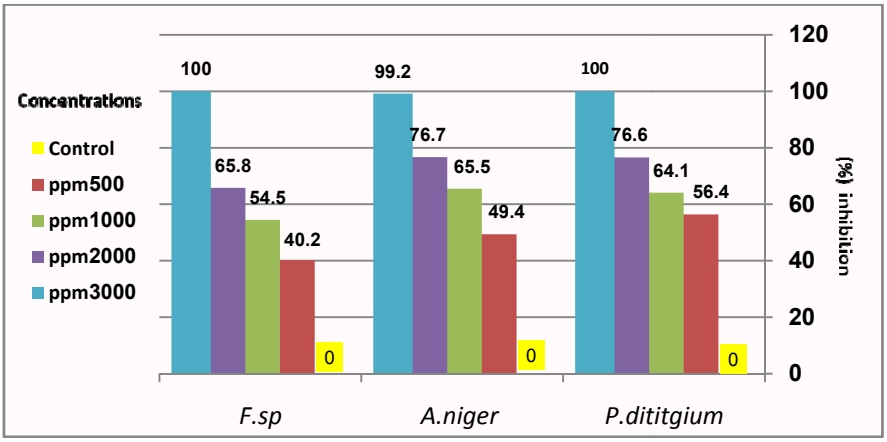

Figure 2 Impacts of Chilli (*Capsicum frutescence* L.) plant extract on colony growth of (cm^2) Penicillium *digitatum* , *Aspergillus niger and Fusarium sp* raised on PDA. Plates were incubated in controlled environment champers maintained at 25C

The impacts of different zinger and chilli 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 (Chili) *Capsicum frutescence and* (Ginger) *Zingiber officinale* L. Plant extracts on colony growth (cm^2) of *Penicillium digitatum , Aspergillus niger and Fusarium sp* raised on PDA.

	Zingiber officinale		*Capsicum frutescence*			
*C*onc ppm	*P.digitatum* *A.niger*		*F.sp* *P.digitatum*		*F.sp* *A.niger*	
	C D(cm^2)	C D(cm^2)	C D(cm^2)	C D(cm^2)	C D(cm^2)	C D(cm^2)
Control	8.467 ±0.120	9.333 ±0.088	6.733 ±0.176	9.033 ±0.033	9.033 ±0.179	7.033 ±0.177
500pppm	4.100 ±0.115	4.133 ±0.115	3.433 ±0.100	3.933 ±0.328	4.567 ±0.189	4.200 ±0.153
1000ppm	2.600 ±0.115	2.500 ±0.066	2.600 ±0.066	2.969 ±0.285	3.100 ±0.100	3.200 ±0.100
2000ppm	2.200 ±0.057	1.966 ±0.088	2.066 ±0.484	2.100 ±0.285	2.100 ±0.115	2.400 ±0.057
3000ppm	1.430 ±0.057	0.800 ±0.176	0.833 ±0.888	0.00 ±0.00	0.033 ±0.333	0.00 ±0.00

CD refers to colony diameter

4. DISCUSSION

The objective of the current research was to study the effect of plant extracts on the mycelia growth of, *Penicillium digitatum*, *Fusarium sp* and *Aspergillus niger* 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). 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 Viudamartos *et al* (2007) as alternative control measures to replace the conventional synthetic pesticides.

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

Aspergillus niger is noted for its carcinogenic alfatoxin 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 *,Artemisia annua ,Eucalyptus globules, Ocimum.sanctum* and *Rheum emodi,* showed significant reduction of the pathogen. Garlic extract had a positive effect on the *Fusarium* inhibition (Anjorin.*et al*,2008) .

In our research, we discovered that zinger and chilli extracts at 3000 ppm showed almost 100% inhibition of the mycelia growth in culture medium.

5. REFERENCES

[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. University of California Publication, California, Pp. 163-195.

[2] Agrios, G.N. 2005. *Plant Pathology*, Academic Press, New York.

[3] 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.Biotechno*l., 7:2402-2406.

[4] 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.

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

[6] 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.

[7] 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.

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

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

[10] 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.

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

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

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

[14] 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.

[15] 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.

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

[17] 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.

[18] 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.

[19] 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.

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

[21] Saxena .and C.S . Mathela, 1996. Antifungal activity of new compounds from *Nepeta leucophylla* and Nepeta clarkei. *Applied Environ Microbiol*.,: 702- 704.

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

[23] 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).

[24] 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.

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