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Research Article

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. (chili) and *Zingiber 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 extract concentrations were more effective than the water extract control in showing antifungal activity ($P<0.05$) against the test pathogens. All 3000ppm concentration from *Capsicum frutescence* L. and *Zingiber 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 of the pathogen by lowering inhibition growth in the storage/transit atmospheres as well as the use of essential natural products as alternative fungicides to control post-harvest diseases in fruit.

Keywords: *Capsicum frutescence* L., *Zingiber officinale* L.**,** antifungal, alcoholic extracts, Malaysia

Introduction

Postharvest disease, such as soft rot of fruit, due to fungal infections cause significant economic losses for the citrus industry during storage, transport and marketing. The predominant pathogens causing the most important postharvest disease of fruit worldwide according to Poppe *et al.* [1], 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 [2]. 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 demonstrate potential as an alternative to synthetic fungicides [3].

Environmentally friendly plant extract agents have shown great potential as alternatives to synthetic fungicides [3, 4]. Recently, the antimicrobial activity of biodegradable and safe higher plant products [5], has attracted the attention of microbiologists. However, the actual use of these products to control postharvest pathogens of fruit, particularly citrus pathogens, is still limited. The purpose of the current research is to test the possibility of using extracts from chili and ginger to control or inhibit post-harvest diseases causing pathogens in citrus fruit.

Materials and Methods

Collection of diseased fruit

Wet markets at Kangar (Perlis) and Georgetown (Penang) were surveyed in December 2010, to observe common post-harvest disease symptoms in oranges, lemons and grapefruit. The prominent symptoms observed were the growth of green, black, white coloured molds on the fruit. Random samples were collected from citrus fruit and brought to the Microbiology Laboratory of the School of Bioprocess Engineering, University Malaysia Perlis for further studies. The fruit 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.

Pathogens

The pathogens identified using 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 fruit 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 [6]. The spores were then refrigerated for further studies and propagation.

Preparation of chili and ginger plants for extractions

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

Preparation of plant extracts

The extraction technique used was a modification of Ruch's method [7]. Up to 50g each of the oven dried and pulverized powered material from chili 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^{o}C for 2 days. The dried powder was stored in small and sterilized 5ml screw-capped glass bottles and were refrigerated (4^{o}C) until further use.

Preparation of plant extract dilutions

The chili and 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 ppm, 1000 ppm, 2000 ppm and 3000 ppm.

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^{o}C. Approximately 5ml of plant extract (500 ppm, 1000 ppm, 2000 ppm, and 3000 ppm), were taken from the Suicide tree, Clove and Mahogany. These 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 [8]. The experiment was performed under aseptic lamina conditions and replicated three times. Approximately 1mL from *P.digitatum, A.niger* and *Fusarium.sp* (conc.1×10^{6} spores/mL) were pipetted on the centre of the amended PDA extracts. The inoculated plates were then incubated at 25^{o}C for 10 days. The petri-dishes inoculated without the extract concentrations served as control. In addition, colony diameter was determined by measuring the average radial growth. The inhibition zone (P), was measured using the formula of Francisco [9]:

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

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

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).

Results and Discussion

Culture PDA media with chili and ginger extract 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 concentrations, 0ppm(control), 500ppm, 1000ppm, 2000ppm and 3000ppm of the plant extracts of *Zingiber 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 concentrations of 500, 1000, 2000 and 3000ppm respectively. *Aspergillus niger* recorded inhibition zones 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 Figure 1 it can also be observed that the 3000 ppm showed the best result in inhibiting the mycelial growth in all the three fungi under study.

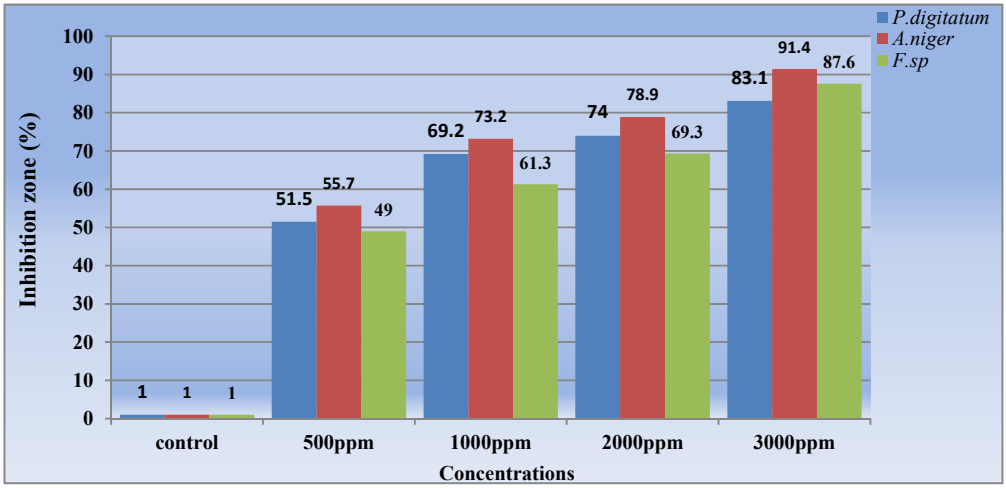

Figure 1. Impacts of ethanolic extract of *Zingiber officinale* expressed as % of inhibition zone on colony growth (cm^2) of *Pencillium digitatum, Aspergillus niger* and *Fusarium sp* raised on PDA and incubated at 25^0C.

Results on the efficacy of chili extract on post-harvest disease in citrus is presented in Figure 2. A similar trend as the ginger extract was observed in its microbial inhibition activity *(P≥0.05),* except that at 3000ppm, all the 3 fungi, namely *Pencillium digitatum, Aspergillus niger* and *Fusarium sp*. recorded almost 100% inhibition zone.

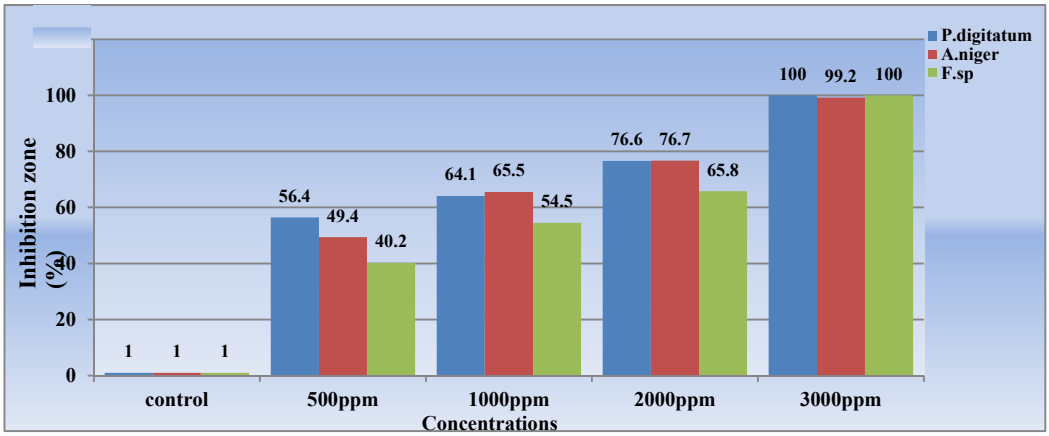

Figure 2. Impacts of ethanolic extract of *Capsicum frutescence* expressed as % of inhibition zone on colony growth (cm^2) of *Pencillium digitatum, Aspergillus niger* and *Fusarium sp* raised on PDA and incubated at 25^0C.

The impacts of different ginger and chili 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. Impact of extracts of *Capsicum frutescence* and *Zingiber officinale* plant extracts on colony growth (cm^2) of *Penicillium digitatum, Aspergillus niger and Fusarium sp* raised on PDA.

	Capsicum frutescence			*Zingiber officinale*		
Treat(ppm)	***P. digitatum*** **CD (cm_2)**	***A. niger*** **CD (cm_2)**	***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

Plant extracts of chili and ginger were used in this study to evaluate the efficacy of botanicals in controlling three fungal pathogens (mycelia growth) of, *Penicillium digitatum*, *Aspergillus niger* and *Fusarium sp* that are pathogens for the post-harvest diseases of citrus as reported by Eckert and Sommer [10] and Adaskaveg *et al*, [11]. These diseases have been known to cause losses of up to 10-30% decrease in crop yield and marketing quality [12].

Other *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 [13]. The plant extracts reported effective against the fungi *Penicillium digitatum* include garlic [6], neem [14], *Withania somnifera* L. and *Acacia seyal* L. [15], mustard and horseradish [16].

Aspergillus niger is noted for its carcinogenic aflatoxin production in diseased plants. Montes-Belmont and Carvajal [17], 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 [18]. Saxena and Mathela [19], in their study on the inhibitory effect of plant extracts on *Fusarium* reported that *Azadirachta indicia* L. (neem), *Artemisia annua* L., *Eucalyptus globules* L., *Ocimum sanctum* L. and *Rheum emodi* L., showed significant reduction of the pathogen. Garlic extract also had a positive effect on *Fusarium* inhibition [20].

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