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Full Length Research Paper

Efficacy of aqueous medicinal plant extracts on growth and citrinin production by *Penicillium citrinum* isolated from rice grains

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In this study we investigated the efficacy of aqueous medicinal plant extracts obtained from *Andrographis paniculata, Cymbopogon citratus, Eurycoma longifolia, Kaempferia galanga* and *Orthosiphon aristatus* on growth and citrinin production by *Penicillium citrinum* isolated from rice grains under *in vitro* (liquid media) conditions. All medicinal plant extracts effectively reduced the growth of *P. citrinum* ranging from 30.1 to 88% and subsequent citrinin production ranging from 42.3 to 91.3% at 10 mg/ml concentration. Of the plant extracts tested, *C. citratus* effectively reduced the growth (88%) and citrinin production (91.3%) by *P. citrinum* at 10 mg/ml concentration followed by *A. paniculata* (76% and 83.69%). Our results showed that these plant extracts can be potentially used to reduce citrinin contamination in rice grains and probably in other food grains.

Key words: Medicinal plants, *P. citrinum*, citrinin, TLC.

INTRODUCTION

Citrinin produced by *Aspergillus* and *Penicillium* spp. has been found as a natural mycotoxin contaminant in food grains and fruit juices (Martins et al., 2002; Allah and Ezzat, 2005; Tangni and Pussemier, 2006; Nguyen et al., 2007; Tabata et al., 2008). Scientific reports showed that a link between citrinin and nephrotoxic (kidney damaging) effects and possibly a carcinogenic effect for humans (Arai and Hibino, 1983; Nguyen et al., 2007). Citrinin enhances carcinogenicity induced by ochratoxin A (OTA) (Kanizawa, 1984; Kumar et al., 2007). Furthermore, citrinin is also embryotoxic, teratogenic and genotoxic (Mossini and Kemmelmeier, 2008). Several strategies have been investigated that can be divided into natural, biological, chemical and physical methods (Reddy et al., 2010) for reduction of mycotoxin contamination in foods. Over the years, efforts have been devoted to search for new antifungal materials from natural sources for food preservation (Galvano et al., 2001; Juglal et al., 2002; Onyeagba et al., 2004; Boyraz and Özcan, 2005; Haciseferogullary et al., 2005).

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The inhibitory effects of plant extracts on mycotoxin biosynthesis have also been examined (Mossini and Kemmelmeier, 2008; Mossini et al., 2009; Singh et al., 2010; Reddy et al., 2009). The extract of *Azadirachta indica* was observed to be a good inhibitor of both growth of *Aspergillus flavus* and *Aspergillus parasiticus*, and toxin production *in vitro* (Bhatnagar et al., 1990). Recently, Mossini and Kemmelmeier (2008) reported more than 90% reduction of citrinin production in *Penicillium citrinum* isolates by aqueous extracts of neem leaf. After this no attempts have been made to test the efficacy of aqueous extracts obtained from medicinal plants other than neem against *P. citrinum* growth and subsequent citrinin production. However, the aim of this study was to test the aqueous extracts obtained from medicinal plants on growth and citrinin production by *P. citrinum* isolated from rice grains.

MATERIALS AND METHODS

Preparation of aqueous extracts

Leaves of five medicinal plants (Table 1) were collected and washed under tap water. Then the leaves were dried in hot air oven at 60°C for 4 days and ground to made powder to pass through 20

Table 1. Ethnobotanical data of the studied plants.

Family	Botanical name	Local name	Used parts	Popular uses
Acanthaceae	*Andrographis paniculata*	Hempedu bumi	Leaves	Diuretic, anti-pyretic, diabetic
Poaceae	*Cymbopogon citratus*	Serai	Leaves	Anti-indigestion, anti-pyretic
Simaroubaceae	*Eurycoma longifolia*	Tongkat ali	Leaves	Anti-malaria, aphrodisiac
Zingiberaceae	*Kaempferia galanga*	Cekur	Leaves	Stomach pains, coughs
Lamiaceae	*Orthosiphon aristatus*	Misai kucing	Leaves	Reduces blood pressure, anti-diabetic

mesh sieve. Ten grams of ground powder were shaken in 100 ml distilled water at 200 rpm for 5 h at room temperature (Razak et al., 2009). The insoluble material was filtered by Whatman No.1 filter paper and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and passed through 0.22 µm membrane filter and stored at -20°C until use.

P. citrinum strain and culture conditions

Citrinin producing *P. citrinum* strain (PC 003) previously isolated from rice grains were used. Spore suspension was prepared by growing the fungi on Petri dishes for 7 days with potato dextrose agar (PDA) containing 50 mg/L of streptomycin. After incubation at 25°C, spores were harvested by adding sterilized distilled (10 ml for each plate) water on each plate. The spore suspension thus obtained was filtered using cheesecloth, and spores were counted using a haemocytometer and brought to a final concentration of 10^5 conidia/ml.

Testing of efficacy of aqueous extracts on *P. citrinum* growth and citrinin production

Twenty millilitre aliquots of potato dextrose broth (PDB) were prepared in 100 ml conical flasks and sterilized at 121°C for 15 min. Various concentrations (2.5, 5.0, 7.5 and 10.0 mg/ml) of aqueous plant extracts were added to cooled liquid broth. A 10 µl amount from suspension contained 10^5 spore/ml of fungus was inoculated in each flask and shaken at 200 rpm for 10 days at 25 ± 2°C. The control contained PDB broth and 10 µl of fungal suspension. The fungal mycelium was harvested by filtrating to separate from liquid culture and then they were dried at 60°C for 24 h. The dry weight of mycelium was determined. All treatments consisted of three replicates, and experiments were repeated twice and determined the averages of the repeated experimental results.

Determination of citrinin produced by *P. citrinum* in liquid media

The culture filtrates (10 ml) obtained from above experiment was used for extraction and estimation of citrinin. Briefly, the citrinin was extracted three times with chloroform (1:1 v/v), pooled and concentrated in vacuo at 40°C using a rotary evaporator. The crude extract was diluted in minimum amount of chloroform (2 ml) and citrinin was estimated by thin layer chromatography (TLC) according to Razak et al. (2009) with minor modifications. Briefly, different volumes (1 to 5 µl) of sample extracts were applied to precoated TLC plates (TLC Silica gel 60 F_{254}, Merck, Germany) along with standard (containing citrinin at 0.5 µg/ml) obtained from Sigma chemical Co. (St Louis, MO, USA). The plates were developed in toluene/ethyl acetate/formic acid (6:4:0.5 v/v) in glass tanks covered with aluminum foil (Mossini and Kemmelmeier, 2008). After development, the plates were dried and observed under long wavelength (365 nm). Citrinin appears as a fluorescent yellow spot. The intensity of the sample spots was compared with that of the standard spot. The citrinin concentration was calculated according to Younis and Malik (2003).

RESULTS AND DISCUSSION

Efficacy of medicinal plant extracts on mycelial growth of *P. citrinum*

The effects of aqueous medicinal plant extracts on mycelial dry weight of *P. citrinum* were presented in Table 2. All plant extracts effectively reduced the mycelial dry weight of *P. citrinum* ranging from 30.1to 88% at 10 mg/ml concentration. Of the plant extracts tested, *Cymbopogon citratus* showed highest growth inhibition ranging from 2.4 to 88% at all concentrations followed by *Andrographis paniculata* ranging from 0.8 to 76%. Other plant extracts showed very less inhibition ranging from 30.1 to 38.8% even at higher concentration (10 mg/ml). These results are in agreement with Mossini et al. (2009) who had reported the significant reduction of *Penicillium verrucosum* and *Penicillium brevicompactum* using neem leaf extract and neem oil. In another study, Mann et al. (2008) reported that the reduction of mycelial growth of *Penicillium* spp. using crude extracts obtained from *Anogeissus leiocarpus* and *Terminalia avicennioides*. This is the first report on testing of aqueous medicinal plant extracts on growth of *P. citrinum* isolated from rice grains.

Efficacy of medicinal plant extracts on citrinin production by *P. citrinum*

The effects of aqueous medicinal plant extracts on citrinin production by *P. citrinum* were presented in Table 3. All plant extracts effectively reduced the citrinin production ranging from 42.3 to 91.3% at 10 mg/ml concentration in liquid media.

Table 2. Efficacy of medicinal plant extracts on growth of *P. citrinum* in liquid media.

Concentration (mg/ml of media)	*Andrographis paniculata*		*Cymbopogon citratus*		*Eurycom longofolia*		*Kaempferia galanga*		*Orthosiphon aristatus*	
	Mdw (mg/ml)	Reduction (%)	Mdw (mg/ml)	Reduction (%)	Mdw (mg/ml)	Reduction (%)	Mdw (mg/ml)	Reduction (%)	Mdw (mg/ml)	Reduction (%)
2.5	240 ± 2.1	0.8 ± 0.1	236 ± 1.1	2.4 ± 0.2	242 ± 1.5	0.0 ± 0.0	241 ± 2.3	0.4 ± 0.1	242 ± 1.2	0.0 ± 0.0
5.0	225 ± 1.2	7.0 ± 0.1	212 ± 2.3	12.3 ± 0.3	240 ± 1.6	2.1 ± 0.1	240 ± 1.9	0.8 ± .1	238 ± 1.8	1.6 ± 0.1
7.5	124 ± 0.9	48.7 ± 1.1	98 ± 1.4	59.5 ± 1.5	198 ± 2.1	18.1± 0.4	189 ± 1.2	21.9 ± 0.6	165 ± 1.0	31.8 ± 0.6
10.0	58 ± 0.2	76.0 ± 1.4	29 ± 0.8	88.0 ± 1.8	167 ± 1.4	30.1 ± 1.0	155 ± 0.7	35.9 ± 1.1	148 ± 2.3	38.8 ± 0.7
Control	242 ± 2.2	0.0 ± 0.0	242 ± 2.2	0.0 ± 0.0	242 ± 2.2	0.0 ± 0.0	242 ± 2.2	0.0 ± 0.0	242 ± 2.2	0.0 ± 0.0

Mdw= Mycelial dry weight.

Table 3. Efficacy of medicinal plant extracts on citrinin production by *P. citrinum* in liquid media.

Concentration (mg/ml of media)	*Andrographis paniculata*		*Cymbopogon citratus*		*Eurycom longofolia*		*Kaempferia galanga*		*Orthosiphon aristatus*	
	AFB1 (µg/ml)	Reduction (%)	AFB1 (µg/ml)	Reduction (%)	AFB1 (µg/ml)	Reduction (%)	AFB1 (µg/ml)	Reduction (%)	AFB1 (µg/ml)	Reduction (%)
2.5	8.4±0.4	8.6±1.0	7.1±0.6	22.8±1.2	9.2±0.3	0.0±0.0	8.7±0.3	5.4±0.6	9.2±0.5	0.0±0.0
5.0	6.5±0.2	29.3±1.2	4.8±0.2	47.8±1.8	8.4±0.3	8.6±0.2	7.8±0.1	15.2±0.8	8.9±0.6	3.2±0.2
7.5	3.2±0.1	65.2±3.2	2.1±0.1	77.1±2.3	7.0±0.2	23.9±1.1	5.8±0.2	36.9±1.2	7.6±0.3	17.3±0.6
10.0	1.5±0.1	83.6±1.9	0.8±0.1	91.3±2.7	5.3±0.1	42.3±0.9	4.2±0.1	54.3±1.1	5.1±0.2	44.5±1.1
Control	9.2±0.2	0.0±0.0	9.2±0.2	0.0±0.0	9.2±0.2	0.0±0.0	9.2±0.2	0.0±0.0	9.2±0.2	0.0±0.0

Among the plant extracts tested, *C. citratus* effectively inhibited the citrinin production by *P. citrinum* ranging from 22.8 tp 91.3% followed by *A. paniculata* ranging from 8.6 to 83.6% at all concentrations tested. Other plant extracts showed less reduction ranging from 42.3 to 54.3% even at higher concentration (10 mg/ml). These results are in agreement with Mossini and Kemmelmeier (2008) who had reported that more than 90% reduction of citrinin produced by *P. citrinum* in liquid media using neem leaf extract at 6.25 mg/ml. Our plant extracts showed that 91.3% reduction of citrinin at 10 mg/ml concentration. After this no attempts have been made to test the efficacy of medicinal plant extracts on citrinin production by *P. citrinum*. This is the first report on testing of medicinal plant extracts on reduction of citrinin production by *P. citrinum* isolated from rice grains.

Conclusion

The overall results of this study showed that aqueous extracts obtained from medicinal plants were effective in reducing growth and citrinin production by *P. citrinum* under *in vitro* conditions in liquid media. So, these plant extracts can be used as a potential source of sustainable eco-friendly botanical fungicides to protect food grains from toxigenic *P. citrinum* and citrinin accumulation under storage conditions. We hope this study will facilitate further screening of various crude plant extracts to find effective novel anti-fungal compounds to control mycotoxigenic fungi and resultant mycotoxins on food grains to ensure food safety and to protect consumer's health.

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