

Inhibition Effect of Betel Leaf Extract on the Growth of *Aspergillus flavus* and *Fusarium verticillioides*

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Abstract

Public concern regarding food safety has increased in recent years as mycotoxins have been reported hazards associated with animal feeds. *Aspergillus flavus* and *Fusarium verticillioides* contaminations in food grains have been reported worldwide. These fungi produce aflatoxin and fumonisin, respectively, that are harmful to humans and animals. Chemicals such as organic acids have been used to control fungi in animal feeds. However, they are unfriendly to users. The experiments were conducted to study the inhibition effect of betel leaf extract on the growth of *A. flavus* TISTR 3366 and *F. verticillioides* TISTR 3175, which were obtained from Thailand Institute of Scientific and Technological Research. An extraction of betel leaf with 95 % ethanol had been carried out. The extract at concentrations of 10, 100, 1000 and 10,000 ppm and the control (without the extract), then, were tested against the growth of these fungi in 10 replicates. The fungi were cultured on potato dextrose agar and incubated at 30°C for 7 days. It was found that the extract at 10,000 ppm completely inhibited the growth of these fungi. The concentrations at 10 and 100 ppm inhibited the growth of the *A. flavus* 5.00 and 6.55 % which were not significantly different, whereas the concentration at 1,000 ppm inhibited this fungal growth at 48.90 %. The growths of the *F. verticillioides* were inhibited 16.51, 22.41 and 33.53 % when treated with the betel extracts at 10, 100 and 1,000 ppm, respectively. These results suggested the high potential inhibition effect of the betel extract on these fungi.

Keywords : betel leaf extract, growth inhibition, *Aspergillus flavus*, *Fusarium verticillioides*

1. Introduction

Betel is a shade loving perennial root climber which belongs to the family Piperaceae. Its scientific name is *Piper betel* L. The leaves contain essential oils including chavicol, chavibitol eugenol, carvacrol, caryophyllene and sitosterol [1]. The essential oils contained in the leaves possess antibacterial antiprotozoan and anti fungal properties [2]. *Aspergillus flavus* can be pathogenic for plants and animals, including humans and domestic animals. The fungus can grow on a broad variety of

feed and food commodities and produces aflatoxin, a toxic and carcinogenic compound [3]. Exposure of livestock to aflatoxin has been found to reduce feed intake, lower daily gain and reduce feed efficiency, which lead to economic loss to the producer [4]. Residues of aflatoxin in animal products pose a threat to human health [5]. The fungus *Fusarium verticillioides* (formerly *F. moniliforme*) is often found in corn and produces a toxic substance, fumonisin. Toxicosis of fumonisin has been found to cause equine

leukoencephalomalacia (ELEM) in horse, pulmonary edema (PE) in swine and esophageal cancer in human [6].

Mold growth controls in feeds include chemical treatments (e.g. ammoniation, organic acid application) and physical treatments (e.g. irradiation, cooling and drying), which are unfriendly to users and cost ineffective. The aim of our research was to control the growth of *A. flavus* and *F. verticillioides* with betel leaf crude extract. The results from this research would of benefit development strategies on mold growth control in feeds which are safe for users and lower the cost.

2. Materials and Methods

2.1 The plant extraction

Fresh leaves of betel were rinsed with distilled water, dried in the air and then finely ground. The leaf powders were then extracted with 95% ethanol (1:5 w/v ratio) at room temperature three times, 72 h each. The mixture was filtered and the solvent was evaporated with a rotary evaporator at 55°C. The resulting extract was placed into the desiccator until the weight was constant.

2.2 Source of fungi and culture conditions

A. flavus TISTR 3366 and *F. verticillioides* TISTR 3175 were obtained from Thailand Institute of Scientific and Technological Research. Before the anti fungal assay was performed, *F. verticillioides* TISTR 3175 was cultured on PDA at 30°C for 7 days whereas *A. flavus* TISTR 3366 was cultured in YM broth and incubated in orbital shaker incubator at 200 rpm and 30°C for 3 days.

2.3 Anti fungal assay and statistic analysis

The betel crude extract was diluted with 100% ethanol. 100 µl of the solution was appropriately incorporated into molten PDA medium (ca. 45°C) in aseptic conditions to have concentrations of 10, 100, 1000 or 10000 ppm in the medium. 100 µl of 100% ethanol was incorporated into the

medium to serve as a control. Into each Petri dish (88 mm in diameter), 20 ml of the medium was distributed.

Circular blocks of mycelia from stock culture of the *F. verticillioides* (5 mm in diameter) were punched using a sterile cork-borer and 100 µl YM broths containing the *A. flavus* were pipeted using a sterile auto pipette and were centrally placed onto the medium incorporated with concentrations of the extract in the petri plate. Ten replicates were maintained for each concentration and fungus. Growth in diameter (in mm) of each fungus was measured at day 7. The data were subjected to analysis variance, and LS means were arrived at using SAS version 9.1.3 [7]. Using the mean values, the percentage of inhibition was calculated using the formula:

$$\% \text{ inhibition} = 100 - \frac{\text{growth in treated}}{\text{growth in control}}(100)$$

3. Result and discussion

Betel leaf extract was sticky and dark green with 9.49 % w/w yield. Antifungal growth of betel leaf extract was quantitatively assessed by colony diameter (mm) and percentage inhibition (Table 1). *A. flavus* grew fastest on the control with the colony diameter at 71.57 mm. Inhibition effect of betel leaf extract on the growth of *A. flavus* was in a dose dependent fashion. As the concentration of the betel extract increased from 10 to 100, 1,000 and 10,000 ppm, the diameter of the colony decreased from 69.01 to 66.88, 36.57 and 0.00 mm, respectively, and percentages of inhibition were 5.00, 6.55, 48.90 and 100.00, respectively. Lewu et al. (2006) [8] reported that acetone and methanol extraction of *Pelargonium sidoides* shoots and roots at 5 mg/ml (5000 ppm) showed more than 50 % inhibition with activity ranging from 52.50 to 82.50 % on *A. flavus*. Trongvanichnam et al. (2007) [9] reported that ethanol extraction of betel leaf at 500 ppm inhibited growth of *A. flavus* at 41 %. Ubalua and Oti

(2008) [10] reported that the combination of the extracts of garlic and *Garcinia kola* at 50 mg/ml (50,000 ppm) demonstrated a remarkable inhibition of pathogens, which included *A. flavus*, on cassava roots after 16 days in storage with 2 % rot. Srichana et al. (2009) [11] reported that mangosteen (*Garcinia mangostana*) fruit hull extracted with 95 % ethanol at 10,000 ppm inhibited growth of this fungus at 57.94 %. However, the recent research found the higher potential inhibition by betel leaf extract, as a concentration of 10,000 ppm completely inhibited growth of this fungus.

As shown in Table 1, *F. verticillioides* colony diameter of the control was biggest at 55.63 mm. Inhibition effect of betel leaf extract on the growth of *F. verticillioides* was also in a dose dependent fashion. As the concentration of the betel extract increased from 10 to 100, 1,000 and 10,000 ppm the diameter of the colony decreased from 46.44 to 43.16, 36.98 and 0.00 mm, respectively and the percentages of inhibition were 16.51, 22.41, 33.53 and

100.00, respectively. Somda et al. (2007) [12] reported neem (*Azadirachta indica*) crude oil had no adverse effect on the growth of *F. verticillioides*, while lemon grass oil (*Cymbopogon citrates*) at a concentration of 2-6 % (20,000-60,000 ppm) inhibited growth of this fungus ranging from 21.40 to 41.30 %. Srichana et al. (2009) [11] reported that mangosteen fruit hull extracted with 95 % ethanol at 10,000 ppm inhibited growth of this fungus at 42.56 %. However, the recent research found that betel leaf extract at a concentration of 10,000 ppm completely inhibited growth of this fungus.

4. Conclusion

Ethanol extracts of betel leaf at 10,000 ppm completely inhibited growth of *A. flavus* TISTR 3366 and *F. verticillioides* TISTR 3175. The results suggested the high potential inhibition effect of the betel leaf extract on these fungi.

Table 1 Growth and percentage inhibition of *A. flavus* and *F. verticillioides* on PDA incorporated with betel leave extract

	Betel leaf extract (ppm)					CV
	0	10	100	1000	10000	
<i>A. flavus</i>						
Growth (mm)	71.57 ^a	69.01 ^{ab}	66.88 ^b	36.57 ^c	0.00 ^d	7.00
Inhibition (%)	0.00 ^d	5.00 ^c	6.55 ^c	48.90 ^b	100.00 ^a	12.88
<i>F. verticillioides</i>						
Growth (mm)	55.63 ^a	46.44 ^b	43.16 ^c	36.98 ^d	0.00 ^e	5.88
Inhibition (%)	0.00 ^e	16.51 ^d	22.41 ^c	33.53 ^b	100.00 ^a	9.02

^{abcde} Means that in a row that does not have a common superscript they differed ($P < 0.05$).

5. References

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