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Original Article

The effect of plant extracts *hymenocrater longiflovus* on *the Fungus Aspergillus Flavus*

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ABSTRACT

The objects of this study concerted to identify the develop *Aspergillus flavus*, *Hymenocrater longiflovus* extracts have been evaluated for their bioactivities on *A. flavus*. In total, 100 samples of nuts were collected from markets of Iraq. Through the study, PDA media were used to isolate *A. flavus*. The results revealed the *A. flavus* was identified in 65% from nuts. *Aspergillus flavus* isolates were further culture on PDA media contain ethanol *H. longiflovus* extracts, and hydroethanol *H. longiflovus* extracts. The results showed the effect of different concentrations of plant extracts on radial mycelial growth of *A. flavus*. Increasing concentration of plant extract significantly reduced the mycelial growth of the pathogen. These results evidently indicate that the plant *H. longiflovus* had inhibitory effect on post-harvest pathogen of nuts in alcoholic extracts of *H. longiflovus* have been reported to have antimicrobial properties.

Keywords: A. flavus, aflatoxin, biosynthesis, Hymenocrater longiflovus, nuts

INTRODUCTION

Contamination of foods and feeds by toxigenic fungi is an important food and feeds safety concern for nuts and other agricultural products. Foods and feeds contaminated with toxigenic fungi, particularly with aflatoxins, can cause sometimes chronic disease, and are associated with increased cancer risk (1). Every year a significant percentage of the world's Nuts are contaminated with hazardous mycotoxins such as aflatoxins. Most countries have established maximum tolerated level for total aflatoxins ranging from 4-20 ng/g (2). Several techniques have been proposed from time to time to prevent the growth of mycelia in toxigenic fungi and reduce the toxin levels, detoxify the toxins in foods and feeds. The use of chemical compounds for control of toxinigenic fungi has always raised concerns about both the environmental impact and the potential health risks related to their use. In addition to this, synthetic compounds appear to be not very successful against the pathogens (3). Nature has been a different source of therapeutic factors for thousands of years and a remarkable number of new drugs have been derived from plant sources (4). Generally around 350,000 plants species of believed to exist, one-third of those have yet to be discovered. Of the one-fourth of million that have been reported, only a portion of them have been investigated based on chemically compounds (5). In order to investigation and identify the effect of plant extract on growth rate of *A. flavus* mycelium, *H. longiflovus* extracts have been evaluated for their bioactivities on *A. flavus*. A series of compounds with antifungal activity against different fungi isolates have been found in different plants (6). These compounds have been used directly or considered as a trial product for the developing of new drugs (7). Christensen and Kolomiets (8) were demonstrated the role of oxygenated lipids from oxylipins in different plants as agents that toxin biosynthesis. The research on mycotoxin biosynthetic pathways revealed the normal factor they share is that they are susceptible to the influence of reactive oxygen species (9). Christensen and Kolomiets were found that some linoleic acid derivatives are able to inhibit toxin synthesis in *A. flavus* (8). Several studies have demonstrated the effectiveness of pharmacological activity in plant extracts (10). Shippamann (12) reported that more than 15,000 flowering plant were considered to be found in Malaysia's rainforests. Carlson (11) presented that around 1.6% of the randomly collected plants were active compared to 15% of the plants presented by the traditional Mayan healer. The antifungal activity of *Moringa oleifera*, *Allium sativum*, *Carica papaya*

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and *Azadrachta indica* on the growth of isolates of *A. flavus* has been investigated (13).

In this study we selected *H. longiflovus* which has been used as traditional drug primarily for treatment of postharvest fungal species. Anti-fungal effect of *H. longiflovus* against toxigenic fungi led to the hypothesis that plant-derived ethanol compounds would have an antifungal effect on *A. flavus*. This hypothesis was supported by studies showing that *H. longiflovus* ethanol compounds exhibit antifungal activities (14).

MATERIALS AND METHODS

Isolation of Aspergillus flavus: Isolation of A. flavus on Potato Dextrose Agar (PDA) medium from nuts by culture the specimens of nuts by streaking on PDA medium and incubation at 35 0C after sufficient incubation, isolated colonies should be visible in the streaked areas and confluent growth in areas of heavy inoculation. *A. flavus*: powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface.

Plant materials and extract preparation: Plant with leaves were washed and rinsed in tap water, dried at 40°C using forced air convention oven dryer and powdered by grinder machine. 12 g of sample was macerated at room temperature in 400ml of 70% ethanol and also 12 g of sample was macerated at room temperature in 400 ml of hydroethanol the beakers are placed on shaker machine for 48 hours. The extract was filtered through filter paper and evaporated to dryness under reduced pressure by pour the extract in petri dishes and placed in the oven for two days to completely dry. The crude extract suspended in 70% ethanol by dissolves 0.5 g of crude extract in 15ml from 70% ethanol and dissolve 0.5 g of crude extract in 15 ml from hydroethanol (15).

Preparation of Culture Media: The PDA culture media were mixed by *H. longiflovus* crude extract according to Tijjani et al. (2014), briefly, 0.5 g of crude extract was dissolved in 15 ml from 70% ethanol, then 2 and 4 ml of obtained solution was suspended in 70% ethanol by dissolve. Then, all *A. flavus* isolates were cultured on PDA medium and incubation at 35 0C. The growth rate of all isolates was examined during 2, 4, and 6 days after incubation.

RESULTS AND DISCUSSION

In total, 100 samples of nuts were collected from markets of Iraq. The results revealed the *A. flavus* was identified in 65% from nuts. In this study *A. flavus* isolates was identified by macroscopic and microscopic characters of isolates (16). Bioactivity of 2 and 4 ml *H. longiflovus* hydroethanol and ethanol extracts showed that all the ethanol plant extracts significantly ($P< 0.05$) reduced the mycelial growth of the *A. flavus* isolates than the control (Table 1). The results showed that 4 ml concentration of *H. longiflovus* hydroethanol extract could reduce the mycelial growth of the *A. flavus* isolate more than 2 ml (Table 1). Investigation on the antifungal properties of *H. longiflovus* on the growth of isolates of *A. flavus in vitro* showed that crude extracts possess some inhibitory components which cause significant reduction in mycelial growth of the fungi. The results also showed the effect of different concentrations of plant extracts on radial mycelial growth of *A. flavus*. Increasing concentration of plant extract significantly reduced the mycelial growth of the *A. flavus* did differ significantly in the reduction of mycelial growth of the pathogen when compared with that of the control. Ebele (17) found that the extracts of some aqueous plant extracts reduced the radial mycelial growth of mycotoxigenic fungi and our study had also confirmed and established the antifungal activity of plants crude extracts, which are interestingly systemic in action and can be used or applied as post-harvest tuber treatment against diseases caused by *A. flavus*. This agrees with earlier reports/works of (18) on the inhibition of growth and sporulation of fungal pathogens.

CONCLUSION

In conclusion, this study had shown that the *Hymenocrater longiflovus* extract used, have the potentials in the protection of nuts against micotoxigenic fungi especially diseases caused by *A. flavus*. Therefore, due to the fact that chemical control of disease is environmentally hazardous and very expensive, this inexpensive, non-hazardous and biodegrable plant material could be used as an alternative way of reducing and controlling on diseases caused by *A. flavus*, to increase nuts production in many developing countries.

Conflicts of interest: There are no conflicts of interest.

TABLE1: EFFECT OF 2 and 4 ml FROM ETHANOL, ETHANOL PLANT EXTRACT, AND HYDROETHANOL PLANT EXTRACT ON GROWTH RATE OF *A. FLAVUS* MYCELIAL

Concentration	Growth rate (mm)[a]			Control (mm)[a]
	Ethanol	Ethanol Plant Extract	Hydroethanol Plant Extract	
2 ml	54.75	57.50	61.5	8
4 ml	26.25	28	38.25	8

[a]Growth rate of *A. flavus* colony after 6 days (mm)

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