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Antifungal Activity of a Combination of Algeria Honey and Starch of Ginger Against *Aspergillus niger*

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Abstract: To evaluate the additive action of ginger starch on the antifungal activity of honey, a comparative method of adding honey with and without starch to culture media was used. *Aspergillus niger* was used to determine the minimum inhibitory concentration (MIC) of four varieties of Algerian honey. In a second step, lower concentrations of honey than the MIC were incubated with a set of concentrations of starch added to media to determine the minimum additive inhibitory concentration (MAIC). The MIC for the four varieties of honey without starch against *Aspergillus niger* ranged between 53% and 57% (vol/vol). When starch was incubated with honey and then added to media, a MIC drop has been noticed with each variety. It ranged between 51% honey (vol/vol) with 4% starch and 47% honey (vol/vol) with 2% starch. The use of ginger starch allows honey benefit and would constitute an additive effect to the antifungal activity of honey.

Key words: Honey · Ginger Starch · Antifungal Activity · *Aspergillus niger*

INTRODUCTION

Aspergillus niger may cause allergic bronchopulmonary disease, invasive aspergillosis or may be a colonizer of natural or preformed cavities of the human body [1, 2]. Resistance of Aspergilli to some clinically used antifungals brings a worrying clinical prognostic in people attacked by aspergillosis [3, 4]. For over fifty years antibiotics have been applied for treating or inhibiting infections. The wide use and sometimes misuse of chemo-antimicrobials in both human and animal medicine has been responsible for the selection of resistant strains [5, 6].

Regarding the increasing clinical importance given to fungi causing infections and the development of drug resistance many scientific studies focusing on the antifungal properties of honey have been carried out [7]. Honey is a saturated solution of sugars, with very low water content [8]. This condition is inhibitory to the growth of bacteria. On the other hand, fungi are generally more tolerant than bacteria to the high osmotic effect [9]. It can be suggested that amylases present in honey originating from bees and pollen are responsible in the hydrolysis of starch chains to randomly produce dextrin and maltose that increase the osmotic effect of honey and consequently increase the antibacterial activity. The importance of starches is long recognized as they are an important source of energy and contribute to the structure and texture of foods [10].

This study was carried out to evaluate the antifungal properties of honey and starch of ginger when used jointly to manage superficial mycoses.

MATERIALS AND METHODS

Honey Samples and Plant Extract: From the 2009 harvest four varieties of honeys of different botanical origin, namely: citrus (V1), jujube (V2), orange (V3) and multi floral (V4) were collected from hives located in western Algeria. All honeys were kept in glass vials, protected from light at temperature of 4°C.

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Rhizome of ginger (*Zingiber officinal*) purchased from local market of Tiaret (Western of Algeria) was peeled and crushed using a hammer mill and then diluted in water. Obtained milk was sieved and sediment separated from the supernatant and washed several times. The deposit obtained was spread out on aluminum foil and dried at 45°C during 48 h. Obtained product was crushed to have starch powder [11] from which various concentrations were prepared and expressed as percentage (%).

Fungal Strains and Inoculums Standardization: *Aspergillus niger* was kindly provided by the Institute Pasteur d'Alger. Strains were maintained by subculture in specific media (Sabouraud agar). Stock suspensions of *A. niger* were prepared from sporulating 7-day-old cultures grown on Sabouraud at 28°C. Colonies were covered with 5 ml sterile distilled water and the surface was scraped with a sterile loop. The mixture of conidia and hyphal fragments was filtered through a 8 mm sterile filter and collected in a sterile tube. This procedure removed the majority of the hyphae, producing inocula composed mainly of spores [12, 13] Turbidity of the final inocula was adjusted to 0.5×10^6-5.0×10^6 spores ml^{-1}.

Minimum Inhibitory Concentration (MIC): Increased concentrations of honey (10-50 % vol/vol) were incorporated into the media to test their efficiency against *A. niger*. Each plate with final volume of honey and media of 5 ml was inoculated and incubated at 37°C for 5 days. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain didn't grow [14]. All MIC values are expressed in % (vol/vol).

Minimum Additive Inhibitory Concentration (MAIC): To evaluate the effect of starch on the antifungal action of honey, a 1 % starch solution was prepared using sterile water. Different volumes from the stock solution were added to a range of honey concentrations lower than the MIC. The same volume of starch solution that has given inhibition with honey is added alone to media as control to check whether or not starch alone has an inhibition effect against *A. niger*. An equivalent volume of water was added to honey instead of starch solution to confirm that inhibition is not due to the dilution of honey. The final volume in each plate was 5 mL. Starch content in media ranged between 1 and 8% (wt/v). Honey and starch as well as honey and water were incubated for 5 days at 37°C before being incorporated into media [14]. Plates were inoculated and incubated at 37°C for 5 days. All inoculations were carried out in duplicates.

RESULTS

The inhibitory action wasn't seen either in the media containing starch only or in media with honey and water. All varieties of honey were effective against the tested strain; without starch of ginger, the MIC of the four

Table 1: Minimum inhibitory concentration (MIC) and Minimum additive inhibitory concentration (MAIC) of tested honeys

		MAIC values % (v/v) honey and starch								Control	
		Starch solution								MIC values % (v/v)	MIC values % (v/v)
	MIC values % (v/v) Honey only	1%	2%	3%	4%	5%	6%	7%	8%	starch only	Water and starch
V_1	55	55	49	49	49	49	49	49	49	0	0
V_2	57	51	51	51	51	51	51	51	51	0	0
V_3	56	50	50	50	50	50	50	50	50	0	0
V_4	53	53	53	53	47	47	47	47	47	0	0

Table 2: Minimum inhibitory concentration (MIC), Minimum additive inhibitory concentration(MAIC) and MIC % of tested honeys

		MAIC% (vol/vol) honey and starch		
	MIC values % (vol/vol) Honey only	Honey %	Starch %	MIC drop %
V1	55	49	2	10.90
V2	57	51	2	10.52
V3	56	50	2	10.71
V4	53	47	4	11.32

varieties ranged between 53 and 57% (vol/vol). When starch was incubated with honey and added to media, the MAIC of the four varieties ranged between 47 % (starch 4%) and 57% (starch 1%) (vol/vol). (Table 1 & 2).

DISCUSSION

Aspergillus niger is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans [15]. Honey is a divine drug quoted in all the scriptures. The therapeutic value of honey was under-scored in various literatures. It occupied a prominent place in traditional medicine. In previous study, Boukraa *et al.* [16] using other varieties of honey, corn starch and the same strain of *A. niger* used in present study, obtained a MIC value ranging between 37 (starch 2, 6%) and 45 (starch 2%). Amylases present in honey were expected to split starch chains to randomly produce dextrin and maltose and probably increase the osmotic effect in the media by increasing the amount of sugars and consequently increase the antifungal activity. As a paradox, the variety which has the lowest diastase number has shown a highest MIC and the variety with the highest diastase number has shown the lowest MIC.

Evidences of amylase in yeast, bacteria and moulds have been reported [17.18.19] many fungi had been found to be good sources of amylolytic enzymes [20]. The *A. niger* group is wide spread with many strains capable of producing amylases[21]. Omemu *et al*. [22] showed that the susceptibility of the raw starches to the crude enzyme of *A. niger* AM07 was significantly dependent on the starch source.

Resistant starch has received much attention for both its potential health benefits and functional properties. It has properties similar to fiber and shows promising physiological benefits in humans, which may result in disease prevention, Sajilata *et al*. [23] *and* Eerlingen *et al*. [24] showed that an increase in resistant starch (RS) yield was observed with high-amylose corn starch. As the final purpose of our future studies is the use of honey and starch to manage superficial mycoses by an increase in osmotic pressure, the use of resistant starch is not adequate in this case. In previous study Boukraa *et al*. [14] using *Candida albicans* instead of *Aspergillus niger* we showed that the additive action of ginger starch was much higher than that with corn starch. In present study, as *A. niger* is considered as a good source of amylolytic enzymes, we expected that the drop in MIC would be better than that with *C. albicans*, but it was not the case, as we obtained a MIC drop with *C.albicans* ranging between 14,2% and 15, 8% while with *A. niger* the MIC drop ranged between 10,5% and 11,5%. It must be précised that the honey varieties tested are the same in the two studies.

CONCLUSION

Honey is one of the natural products widely used to treat infections that resist conventional drugs. But its price makes it an unaffordable substance in developing countries. Our results show that adding starch to honey could contribute to reducing the quantity of honey to be used without losing the expected effect. Further studies must be carried to understand witch starch for witch mould would be used.

REFERENCES

1. Young, R.C., J.E. Bennett, C.L. Vogel, P.P. Carbone and V.T. DeVita, 1970. Aspergillosis, the spectrum of disease in 98 patients. Medicine. 49: 147-173.
2. Londero, R.T. and J.M. Guadalupe-Cortés, 1990. Aspergiloses pulmonares. J Pneumol., 16: 78-90.
3. Canuto, M.M. and F.G. Rodero, 2002. Antifungal drug resistance to azoles and polyenes. Lancet. Infect. Dis., 2, 550-563.
4. Curtis, L., L. Conroy, S. Coli, K. Baker, C.H. Our, R. Hershow, F. Norlock-Cruz and P. Scheff, 2005. Aspergillus surveillance project at a large tertiary-care hospital. J. Hosp. Infect., 59: 188-196.
5. Desselberger, V., 2000. Emerging and re-emerging infectious disease. J. Inf. Dis., 40: 3-15.
6. Georgopapadakou, N.H., 2002. Infectious diseases 2001: drug resistance, new drugs. Drug Res., 5: 181-191.
7. Koc, A.N., S. Silici, F. Kasap, H.T. Hörmet-Oz and H. Mavus-Buldu, 2008. Antifungal activity of Turkish honey against Candida spp: and Trichosporon spp an *in vitro* evaluation. Med. Mycol., 2: 1-6.
8. Anonymous, 1988. From Natures Food Industry. Information Brochure, 2. National Honey Board Honey, USA.
9. Molan, P.C., 1992. The antibacterial activity of honey: 1. the nature of the antibacterial activity. Bee World, 73: 5-28.
10. Hersalg, L. and J. Montenegro, 1982. Traitement des plaies suppurées par application de Saccharose. Nouv. Press. Méd., 11: 940.

11. Amani, N.G., F. Aboua, D. Gnakri and A. Kamenan, 1993. Etude des propriétés physico-chimiques de l'amidon de taro (Xanthosoma sagittifolium). IAA, 3: 136-143.
12. Petrikkou, E., J.L. Rodrıǵguez-Tudela, M. Cuenca-Estrella A. Goǵmez, A. Molleja and E. Mellado, 2001. Inoculum standardization for antifungal susceptibility testing of filamentous fungi pathogenic for humans. J. Clin Microbiol., 39: 1345-7.
13. Santos, D.A., M.E.S. Barros and J.S. Hamdan, 2006. Establishing a method of inoculum preparation for susceptibility testing of Trichophyton rubrum and Trichophyton mentagrophytes. J. Clin Microbiol., 44: 98-101.
14. Boukraa, L., B. Hama and M. Ahmed, 2008. Synergitic action of starch and honey against *Aspergillus niger* in correlation with diastase number. Mycoses, 51: 520-522.
15. Perfect, J.R., G.M. Cox, J.Y. Lee, C.A. Kauffman, L. De Repentigny, S.W. Chapman, V.A. Morrison, P. Pappas, J.W. Hiemenz and D.A. Stevens, 2001. Mycoses Study Group, Clin Infect Dis., 33: 18241833.
16. Boukraa, L., B. Hama and M. Ahmed, 2008. Synergitic action of starch and honey against *Aspergillus niger* in correlation with diastase number. Mycoses, 51: 520-522.
17. Akpan, I., M.O. Bankole, A.M. Adesemowo and G.O. Latunde- Dada, 1999b. Production of amylase by A. niger in a cheap solid medium using rice bran and agricultural materials. Trop. Sci., 39: 77-79.
18. Adebiyi, C.A.B. and J.A. Akinyanju, 1998. Thermophillic amylase producers from the soil. Nigerian J. Sci. Technol., 11(1): 30-38.
19. Buzzini, P. and A. Martini, 2002. Extracellular enzymatic activity profiles in yeast and yeast - like strains isolated form tropical environments. J. Appl. Microbiol., 93: 1020-1025.
20. Abe, J., F.W. Bergmann, K. Obeta and S. Hizukuri, 1988. Production of the raw starch digesting amylase of *Aspergillus spp*. K-27. Appl. Microbiol. Biotechnol., 27: 447-450.
21. Manno, V. and M. Pekka, 1989. Microbial Amylolytic enzymes. Critical Rev. Biochem. Mol. Biol., 24: 329- 335.
22. Omemu, A.M., I. Akpan, M.O. Bankole and O.D. Teniola, 2005. Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AM07 isolated from the soil. African J. Biotechnol., 4(1): 19-25.
23. Sajilata, M.G., S. Rekha Singhal and R. Pushpa Kulkarni, 2006. Resistant Starch- A Review. Comprehensive Reviews in Food Science and Food Safety, 5(1): 1-17.
24. Eerlingen, R.C., I. Van Den Broeck, J.A. Delcour and H. Levine, 1994. Enzyme resistant starch. Influence of sugars on resistant starch formation. Cereal Chem., 6(70): 345.