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.
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 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 × 10^5-5.0 × 10^6 spores ml^-1.

**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 × 10^5-5.0 × 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>
<thead>
<tr>
<th>MIC values % (vol/vol)</th>
<th>Honey only</th>
<th>Starch solution</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>Honey only</td>
<td>Starch solution</td>
<td>Honey only</td>
</tr>
<tr>
<td>V1</td>
<td>55</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>V2</td>
<td>57</td>
<td>51</td>
<td>51</td>
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<tr>
<td>V3</td>
<td>56</td>
<td>50</td>
<td>50</td>
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<tr>
<td>V4</td>
<td>53</td>
<td>53</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>MIC values % (vol/vol)</th>
<th>Honey only</th>
<th>Starch %</th>
<th>MIC drop %</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>55</td>
<td>2</td>
<td>10.90</td>
</tr>
<tr>
<td>V2</td>
<td>57</td>
<td>2</td>
<td>10.52</td>
</tr>
<tr>
<td>V3</td>
<td>56</td>
<td>2</td>
<td>10.71</td>
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<tr>
<td>V4</td>
<td>53</td>
<td>4</td>
<td>11.32</td>
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</tbody>
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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]. Omemlu 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


