Additive action of honey and starch against *Candida albicans* and *Aspergillus niger*

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Summary A comparative method of adding honey to culture media with and without starch was used to evaluate the action of starch on the antifungal activity of honey. The minimum inhibitory concentration (MIC) expressed in % (v/v) for two varieties of honey without starch against *Candida albicans* was 42% and 46%, respectively. For *Aspergillus niger* the MIC without starch was 51% and 59%, respectively. When starch was incubated with honey and then added to media the MIC for *C. albicans* was 28% and 38%, respectively, with a starch concentration of 3.6% whereas the MIC for *A. niger* was 40% and 45%, with a starch concentration of 5.6% and 5.1% respectively. This study suggests that the amylase present in honey increases the osmotic effect in the media by increasing the amount of sugars and consequently increasing the antifungal activity.

Key words Antifungal, *Aspergillus, Candida*, Honey, Starch

Acción aditiva de la miel y el almidón frente a *Candida albicans* y *Aspergillus niger*

Resumen Se ha evaluado la acción del almidón en la actividad antifúngica de la miel mediante un método comparativo añadiendo miel a medios de cultivo con y sin almidón. La concentración mínima inhibitoria (CMI) expresada en % (v/v) para dos variedades de miel sin almidón frente a *Candida albicans* fue del 42% y 46% respectivamente. Para *Aspergillus niger* la CMI sin el almidón fue del 51% y 59% respectivamente. Cuando se incubó el almidón con la miel antes de añadir al medio, la CMI para *C. albicans* fue del 28% y 38% respectivamente con una concentración de almidón del 3,6% mientras que la CMI para *A. niger* fue del 40% y 45% con una concentración de almidón del 5,6% y el 5,1% respectivamente. Se sugiere que la presencia de amilasa en la miel aumenta el efecto osmótico en los medios aumentando la cantidad de azúcares y por consiguiente aumenta la actividad antifúngica.

Palabras clave Antifúngico, *Aspergillus, Candida*, Miel, Almidón

Both systemic and cutaneous fungal infections are major problems for immunosuppressed individuals. These infections often become difficult to manage with the emergence of multi-drug resistant strains [11]. Honey is a natural product that has been used for its antifungal activity [6], and its antimicrobial properties have been extensively reviewed [7]. Honey has potent antibacterial activity and is very effective in both preventing and clearing wound infections [1]. Unlike honey, starch has been incorporated into microbial media to stimulate their growth, and has not therefore demonstated inhibitory activity [14]. One of the characteristics that sets honey apart from all other sweetening agents is the presence of enzymes. The enzyme content of honey varies according to the floral source, the length of storage, and exposure to high temperatures [13]. The antibacterial activity of honey is mostly due to hydrogen peroxide, continuously produced by glucose oxidase action when honey is diluted [4]. The antifungal activity of honey is thought to be attributed to the high concentration of sugars and low content of water [7]. Therefore, it is expected that adding starch (the substrate of diastase) to honey will subsequently increase the antifungal effect of honey. This study was carried out to evaluate the antifungal properties of honey when used to manage superficial mycoses, and to explore the novel concept that starch, normally considered a microbial nutrient, may actually enhance the antifungal properties of honey.

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Methods

Two multi-floral honey samples (A and B) were analysed. Both samples were obtained directly from beekeepers in different regions of Algeria during the year 2005. The two fungal strains tested were a *Candida albicans* that had been isolated from a dog suffering from otitis media, and a bovine *Aspergillus niger* strain isolated from an animal seen at a local veterinary hospital with pneumonic aspergillosis. Strains were maintained and renewed each week by subculture in specific media (YPGA: yeast peptone glucose agar). Concentrations of honey between 40% and 60% (v/v) were incorporated into media to test their efficacy against fungi. The final volume of honey and media in each plate was 5 ml. The plates were incubated at 37 °C for 48 h and 5 days for *C. albicans* and *A. niger,* respectively. The minimum inhibitory concentration (MIC) was determined by finding the plates with the lowest concentration of honey on which the strain failed to grow. All MIC values are expressed in % (v/v). Sterile water was used to prepare a stock solution of 10% of starch. Varying volumes of this solution were added to a range of honey concentrations below the MIC to evaluate the effect of starch on the antifungal action of honey. The volume of starch solution that demonstrated inhibition with honey was added to the media as a control to check whether or not starch alone has an inhibition effect against fungi. An equivalent volume of water rather than starch was added to honey to confirm that inhibition was not due to the dilution of honey. The final volume in each plate was 5 ml. Honey and starch as well as honey and water were incubated for 24 h at 37 °C before being incorporated into media. This allowed the diastase present in honey to act against starch. Plates were inoculated and incubated at 37 °C for either 48 h or 5 days for *C. albicans* and *A. niger,* respectively. All inoculations were carried out in duplicate with most MIC results being the same. Where there were differences between the replicates the MIC is shown as a range. Statistical analysis was performed using Statistica® software.

Table 1. Additive MIC values between starch and variety A of honey against *Candida albicans*.

Honey % in media (v/v)	40	35	30	28
Starch % in media (w/v)	1.2	2.4	3.1	3.6

Table 2. Additive MIC values between starch and variety B of honey against *Candida albicans*.

Honey % in media (v/v)	44	43	42	41	40	39	38
Starch % in media (w/v)	2.0	2.2	2.3	2.5	2.8	3.2	3.6

Table 3. Additive MIC values between starch and variety A of honey against *Aspergillus niger*.

Honey % in media (v/v)	49	48	47	45	40
Starch % in media (w/v)	0.5	0.9	2.8	4.4	5.6

Table 4. Additive MIC values between starch and variety B of honey against *Aspergillus niger*

Honey % in media (v/v)	57	56	55	50	45
Starch % in media (w/v)	0.9	3.0	4.0	4.8	5.1

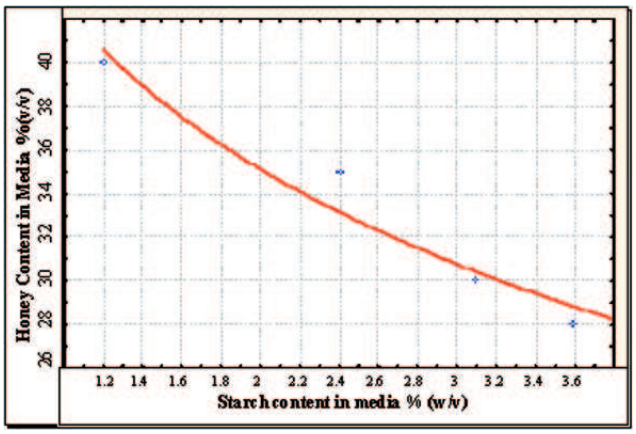

Figure 1. Isobologram representation of overadditive action between starch and variety A of honey against *Candida albicans.*

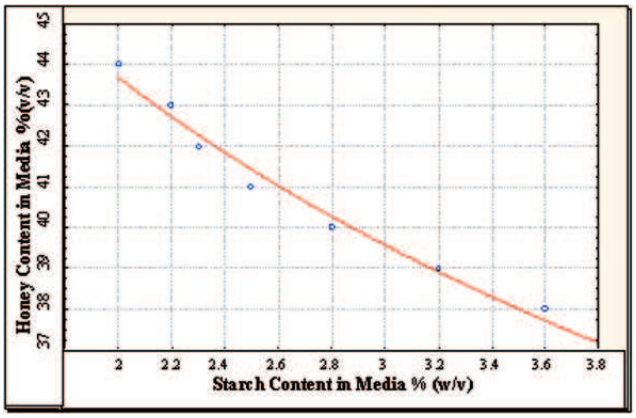

Figure 2. Isobologram representation of overadditive action between starch and variety B of honey against *Candida albicans.*

Results and discussion

Both varieties of honey were effective against both tested strains. Without starch, the MIC of variety A of honey was 42% (v/v) and for variety B was 46% (v/v) against *C. albicans*. For *A. niger* the MIC of variety A of honey without starch was 51% (v/v) and for variety B was 59% (v/v). When starch was incubated with honey and then added to media, the MIC obtained with *C. albicans* was 28% (v/v) for variety A of honey and 38% (v/v) for variety B with a starch concentration of 3.6% (w/v) (Tables 1 and 2). With *A. niger* the MIC was 40% (v/v) for variety A of honey with a starch concentration of 5.6% (w/v) but for variety B of honey the MIC was 45% (v/v) with a starch concentration of 5.1% (w/v) (Tables 3 and 4). The inhibitory action was seen neither in the media containing starch only nor in media with honey and water.

Candida albicans is a fungus which can grow on warm and moist surfaces causing superficial diseases such as oral and vaginal thrush and chronic mucocutaneous candidiasis [9]. The usual route of *A. niger* infection is inhalation but infection sometimes follows local tissue invasion, through surgical wounds or contaminated intravenous [5]. Ophthalmic mycoses are also incited by these agents. Rubbery concretions occur in the presence of infections due to species of *Candida*, whereas brown or black debris may be seen in infections due to *A. niger* [2]. A recent study in China has reported that *Aspergillus* isolates represented 17.3% in fungal ocular pathology [12].

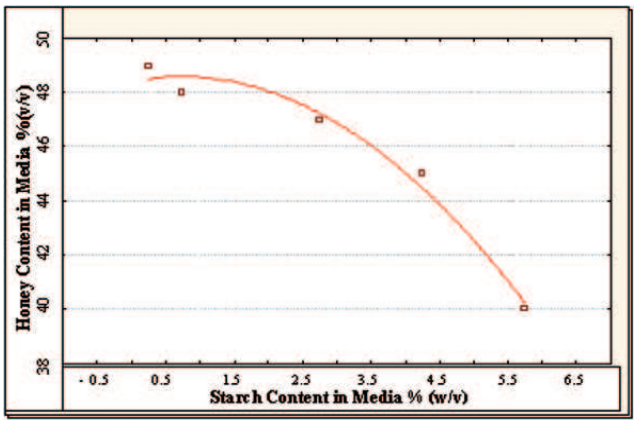

Figure 3. Isobologram representation of underadditive action between starch and variety A of honey against *Aspergillus niger.*

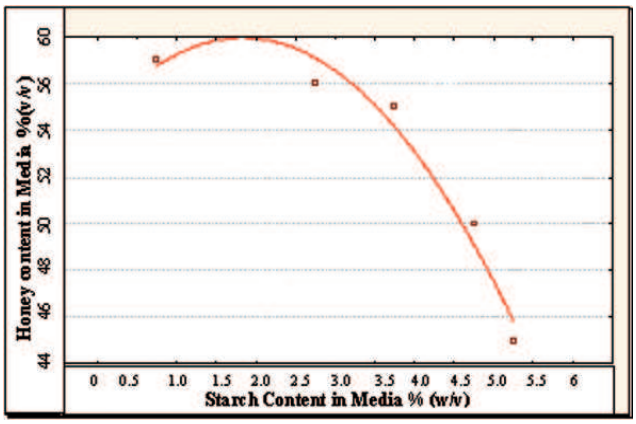

Figure 4. Isobologram representation of underadditive action between starch and variety B of honey against *Aspergillus niger.*

Honey is a known natural antimicrobial medicine [7] used especially for treating infections that are resistant to conventional drugs [1,8]. With the exception of glucose oxidase, no honey enzymes have been associated with its therapeutic properties [10]. The main honey substances are sugars, which by their osmotic effect exert an antibacterial action [7]. Adding starch to honey has shown a significant decrease in the MIC for the two varieties of honey against the tested strains. Figures 1 and 2 represent isobologrames of overadditive action between starch and honey whereas figures 3 and 4 show an underadditive action between honey and starch. The inhibitory action was seen neither in the media containing starch only nor in media with honey and water. As amylases originating from bees and pollen are present in honey, it is presumed that they split starch chains to randomly produce dextrin and maltose, thereby increasing the osmotic effect in the media and consequently increasing the antifungal activity. Adding starch to media containing honey may play an aditive role in this activity. These studies suggest the potential use of starch with honey for greater efficacy when treating topical infections due to *Candida* and *Aspergillus* in both human and animals. Neither honey nor starch have adverse effects on tissues, so they can be safely used on wounds and inserted into cavities and sinuses to clear infection. A clinical trial using a mixture of honey and starch to treat candidiasis and aspergillosis would be necessary to validate these findings.

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