

INTERRELATIONS OF DRYING HEAT AND SURVIVAL OF DIFFERENT FUNGAL SPORES WITHIN THE TABLETS FORMULATION

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Abstract

The level of temperature plays an important role in the survival of the microorganisms, the different species of molds must grow and develop at any temperature their environment is. However, high temperature for a certain period will cause the death of microorganisms.

In this study the objective is to investigate the interrelations of drying heat (55 °C for 30 minutes) used in tablets manufacture on the survival of different fungal spores (*Aspergillus flavus* and *Penicillium spp.*) at different contamination levels (10², 10⁴ and 10⁶) (spores/g) within the prepared tablets.

The results showed that the drying heat caused the inactivation of about 32% of *Aspergillus flavus* spores and 36.6 % of *Penicillium spp.* spores within the granules. In addition, the level of inactivation decrease with increasing the contamination level, especially with 10⁶ (spores/g) which may be related to the possibility of mutation that lead to enhance the resistance toward heat.

Key words : Tablets manufacture; drying heat; fungal spores survival.

INTRODUCTION

The effects of heat on fungi depend on many factors, including the genus, species and strain of the fungus, the amount of available water, kinds of nutrients, and many other environmental factors. Of course, temperature is also a crucial factor. Most of the research on temperature relationships for the fungi has been done in the industry where heat is commonly used to prevent fungal growth. Much of this research involves wet heat, which is more effective than dry heat [1].

Temperature is extremely important for the growth of Fungi. In initial stages, the fungi requires high temperature but less light. It is observed that for ever 10 °C increase in temperature, the growth rate of fungi doubles (during the initial stages). However, after a certain stage, temperature must be kept moderate, as high temperature would kill the Fungi [2].

Fungi can be divided into groups according to temperature requirements for optimal growth. Note that these requirements are dependent on water availability and nutrients, and are measured under carefully controlled conditions. These same terms are used for bacteria, but the temperature ranges differ.

There are also categories defining the ability of the fungi to withstand different temperature regimes. In this case, we have psychrotolerant and thermo-tolerant fungi, indicating that growth can occur at either low or high temperatures, but is not optimal. Thus, some mesophilic fungi may be able to grow or at least survive at either low

or high temperatures, depending on the genetics of the strain and other environmental conditions [1].

While the temperature extremes are of great importance in determining the survival and distribution of fungal species in nature, the effect of temperature upon enzyme activities is of great interest to the experimentalist and to the fungi growth. In the linear phase of growth, for each 10 °C increase in temperature, the growth rate double (i.e., The Q₁₀ is 2). Obviously, this cannot go on indefinitely, for high temperature inactivates enzymes. In some cases, it has been shown that the failure to grow at high temperature was the result of inability to synthesis a required vitamin and the growth of the fungus would take place at higher temperature if that vitamin were supplied in the medium [2].

Another form of temperature tolerance lies in the spores, which can often withstand temperature extremes, and germinate when conditions return to normal. Some fungi that are normally mesophilic have spores that require heat to stimulate germination. Temperature tolerance is strongly tied to the amount of water so that wet heat is much for effective at damaging spores than dry.

The effects of heat on fungi are related to the chemical reactions within the fungal cells. For optimum growth, temperatures must be in a range that allows the most efficient progression of the chemical reactions necessary for growth.



As temperatures progress above the optimum temperature, the chemical reactions occur less efficiently, and growth slows.

Eventually, the temperature can reach a point where growth stops, and cell components begin to be actually damaged by the heat. Enzymes are proteins that change structurally when heated to their limit of tolerance. Likewise, membranes, which contain lipids, change in structure, and their function of protecting and regulating the internal environment of the cell becomes compromised.

Most fungi are mesophilic, and have growth optima within the temperature range that people find comfortable. This is why so many fungi appear when moisture enters our homes, schools, and work environments. Because of air conditioning and heating, mesophilic fungi flourish in occupied environments in all climates. However, the fungal species that are abundant outdoors may vary considerably from one climate to another.

In hot dry climates, fewer species of fungi are present, both because of the lack of water and the high temperatures. Thus, thermophilic and xerophilic (dry tolerant) fungi are likely to be more abundant than in cooler wetter environments. In tropical and subtropical places where both heat and moisture are present, thermophilic and thermo tolerant fungi with mesophilic water requirements tend to be abundant. The incidence of fungal infections (including sinus infections) tends to be higher in these areas in part because the fungi that can withstand human body temperatures are more abundant than in temperate climates.

Finally, continental climates that tend to swing from hot humid to cold dry conditions have a few overwhelmingly dominant fungi (e.g., *Cladosporium* species) with other mostly mesophilic fungi filling in the gaps. Of course, this is an over-simplification, especially since, as mentioned many times, an array of factors are necessary for optimal growth of all kinds of fungi. Also, fungi live in microenvironments that may have very different temperature/water conditions than are represented by climate [1].

MATERIAL AND METHOD

The effect of the drying heat that used during wet granulation method on the percentage of survival of the dried fungal spores of *Aspergillus flavus* and *Penicillium spp.* was studied.

Materials

NaOH: The Pvc Co., Ltd. Of Shandong Xinlong Group; Isopropyl alcohol: Shenyang Jin Yi Lai Chemical Co., Ltd. China; Polysorbate 80: Great Chang Import & Export Co., Ltd. of Shandong, China; Glycerin: Jiangsu Clear Land Daily Chemicals Co., Ltd. China; Chloroform: Palvi Powertech Sales PVT Ltd. India; Normal saline: Ateco Pharma. Egypt.

Universal-neutralization liquid (U.N.L) [3]. (Solution used for preservative neutralization): Soya lecithin: 3.0 gm; Polysorbate 80: 30.0 gm; Sodium thiosulfate pent hydrate: 5.0 gm; L. histidin: 1.0 gm; Peptone: 1.0 gm; Sodium chloride: 8.5 gm; Disodium hydrogen phosphate dehydrate: 4.5 gm; Potassium dihydrogen phosphate: 1.5 gm; Purified water: 1000 ml.

Culture media

Tryptic soy broth (Difco, USA); Sabouraud dextrose agar (Becton and Dickinsons, USA); Rose Bengal agar and Tellurite broth were provided by (IDG, UK); Nutrient broth, Nutrient agar and McConkey broth were all from Merck Chemical Co. (Darmstadt, Germany).

Instruments

Incubator: Memmert (model IFE 400), Germany; Hot Air Circulation Drying Oven: Tiantai Pharmaceutical Machinery Factory (type RXH-4P), China; Horizontal Laminar flow cabinet: Technico (type THFS 222), India; Cooled orbital incubator: Gallenkamp Cooled Orbital Incubator Lancashire,, United Kingdom; Autoclave: Technico (T01/AV – Vertical) India; Balance: Adam equipments (type PGW), UK; Water bath: Memmert (model WPE 45), Germany; Oven: Memmert (model VO 500), Germany; Colony counter: Gupta Agencies (type GLMS 9020), India; Vortex shakers: Heidolph-Group (type multi reax), kelheim, Germany; pH- meter: BOECO (model BT-600), Germany; Centrifuge: DJB Labcare Limited (Hettich EBA20 Portable Centrifuge), UK; Blender: Clarkson Laboratory (type UTI-4AL) USA; Rotary evaporator: BUCHI (Rotavapor type R-210/R-215), Flawil, Switzerland; Humidity chamber: Memmert (model HCP 246), Germany.

Method

Tablets formulations were contaminated with *Aspergillus flavus* and *Penicillium spp.* dried fungal spores in three contamination level (10^2 , 10^4 and 10^6) (spores/g). During tableting process (in this study it was wet granulation method), water was used as a binder, so high temperature (50-70 °C), for long period (30 minutes) required to dry the granules by using hot air try oven [4]. The number of killed spores was determined immediately after finishing the drying process.

Results and discussion

Results indicate that, temperature of 55 °C for 30 minutes killed 34% of the dried fungal spores of *Aspergillus flavus* when the contamination level was 10^2 and 32% of the dried fungal spores of *Aspergillus flavus* when the contamination level was 10^4 and 30% when the contamination level was 10^6 of the dried fungal spores of *Aspergillus flavus*. While the percentage of the killed microorganisms (*Penicillium spp.*) were 41, 37 and 32% when the contamination level of the dried fungal spores

of *Penicillium spp.* were 10^2 , 10^4 and 10^6 (spores/ gram) respectively (table 1).

These high temperatures for certain period inactivate some of the inoculated fungal spores in respect to the geographic location of the fungal spores. The

contaminants in our study were inoculated into the raw material, another word it is presented inside the granules and protected to some extent from the drying heat [3].

The survival of the microorganisms at 55 °C for 30 minutes were as the following. (table 1, figure 1).

Table (1): The percentage survival of the contamination spores after exposing the granules to 55 °C for 30 minutes

Types of fungal spores	Concentration of fungal spores	Percentage of killing at 55 °C for 30 minutes
<i>Aspergillus flavus</i>	10^2	34
	10^4	32
	10^6	30
<i>Penicillium spp.</i>	10^2	41
	10^4	37
	10^6	32

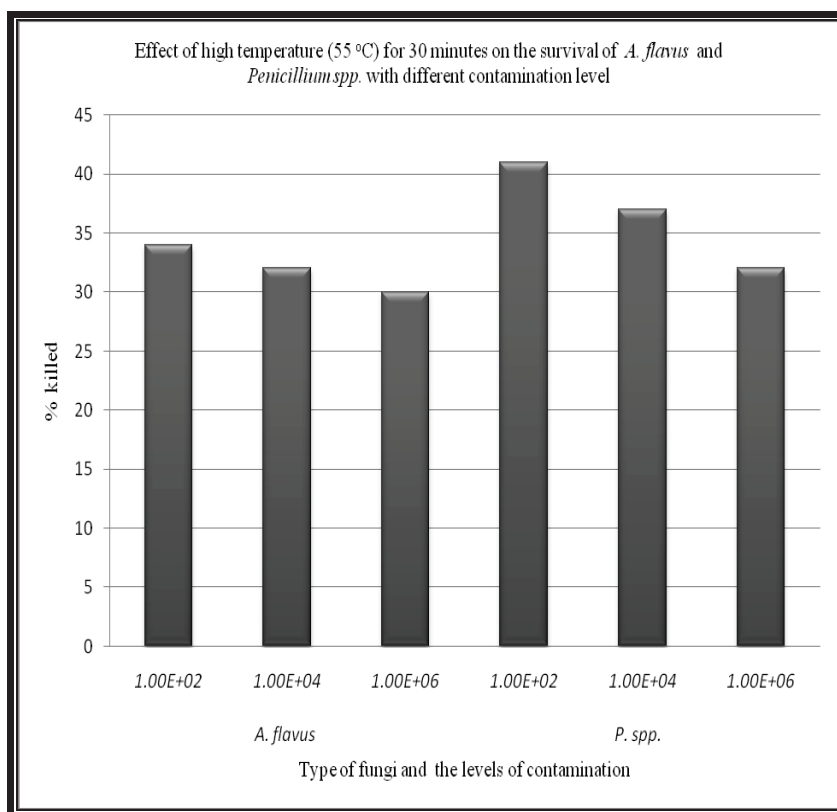


Figure (3): The percentage survival of the contamination spores after exposing the granules to 55 °C for 30 minutes.

CONCLUSION

Our results showed that a temperature of 55 °C for 30 minutes killed a mean of 32% from the total fungal contaminating dried spores' levels of *Aspergillus flavus* within the tablets and approximately 37% from the total fungal dried spores of *Penicillium spp.* within the tablets. These observations support the hypothesis which said that the inactivation of fungal spores during tableting is related to the heat that generated rather than shearing force; therefore, drying heat has the ability to inactivate

fungal spores in different degree depending upon heat sensitivity of that particular type of fungal spores [5].

The percentage of killed spores decrease with increasing the contamination level specially a level of 10^6 (spores per gram) with the two different fungi, this result indicate that at high level of contamination 10^6 (spores per gram), there is a chance for mutation and enhance microbial resistance for heat, in the previous studies they didn't used such high level; therefore, they didn't observed such results [6,7].



Previous work [8] indicate that, most of the vegetative organisms, such as *Escherichia coli* and *Staphylococcus aureus*, were totally inactivated in such process, but in this case we have fungal spores that in which are more resist to drying heat [9], this resistance can in part be attributed to their lack of hydration and low metabolic activity but also to their small size.

The effect upon microorganism of drying might be to some extent predictable by their tolerance to reduce water activities. In general, moulds will tolerate lower water activities than bacterial cells [10].

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