Effect of Some Storage Conditions upon the Survival of Some Fungal Spores

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Abstract

Folic acid and multivitamin tablets containing Aspergillus flavus Penicillia spp. and Cladosporia spores were prepared at a compression pressure of 148 MN/m^2 and stored at 35°C under different relative humidifies (75,85, and 95)% within air tight containers, to study the effect of storage condition on them, as well as ,the estimation of the microbial level of the raw materials intended to be used in the two kinds of tablets . Result showed that some raw materials derived from natural origin were heavily contaminated with microorganism compared to that of synthetic origin ,the results also indicated the effect of relative humidity , types of fungal spore , and the hygroscopic nature of exicpient upon survival. Multivitamin tablets showed more survival than folic acid tablets and this is due to the presence of more nutrients. No aflatoxin was obtained from both multivitamin and folic tablets at 35°C temperature; this is due to the temperature which is not an optimum temperature for aflatoxin B_1 production.

Key words: ***Storage conditions of tablet, fungal spores*.**

الخلاصة

تم تحضير حبوب حامض الفوليك ومحبوب مجموعة من الفيتامينات تحت ضغط 148 ميكا نيوتن / م2 وتم خزنها في درجة حرارة 35°م ورطوبة نسبية مختلفة كما تم حساب التلوث الميكروبي للمواد الخام المستخدمة في تحضير نو عين من الحبوب ولقد بينت النتائج أن المواد الأولية (الخام) ذات الأصل الحيواني او النباتية التي تدخل في الصناعة المستحضرات الصيدلانية أكثر ملوثة من المواد الأولية ذات الأصل الصناعي ماعدا التي تبين من البحث احتوائها على بكتريا مرضية .. وقد تبين من خلال البحث إن بعض الأنواع قد قاومت عملية الكبس ولذلك دعت الى دراسة ظروف بدت مختلفة وتأثيرها على هذه الفطريات وقد أظهرت النتائج ان الحبوب التي تحوي مجموع من الفيتامينات تظهر بنسبة اعلى من التلوث بسبب ما تحويه من مواد غذائية وان الفطر اسبارجلاس أظهر على نسبة للتلوث مقارنة ببقية الفطريات ولم يسجل اي نسبة للافلاتوكسين وهذا يعود إلى إن درجة 35°م غير ملائمة لإنتاج افلاتوكسين ب 1.

Introduction

The microbiological quality of non-sterile pharmaceuticals (tablets) is largely determining by the microbial contamination of a raw materials. The effect of the manufacturing process and the fate of contaminating microorganisms during storage. Several infection outbreaks which would be traced back to the use of heavily contaminated raw materials of natural origin have been reported [(1) (2) (3) and (4)] .During manufacturing the viability of microbial cells can be significantly affected by the drying process of granules [(5)] and by the actual compaction [(6) (7)] .The availability of water probably plays an important role. As long as tablets are stored under dry conditions spoilage due to growth of micro organisms is unlikely to occur [(8)]. However, in regions with a hot and humid conditioned, growth of contaminating micro-organisms cannot be excluded. More ever ,in such countries pharmaceutical preparations are frequently stored under uncontrolled conditions and may be dispended in non protective packaging or even without any packaging at all. Few studies that investigate the effect of storage on the microbiological quality of tablets [(9) (10) (11)] ,but little informations are available upon the fungi as contaminants in pharmaceutical industries and possible toxicogenic power [(13) (15) (16)] .The aim of this study was to investigate the effect of storage under different conditions on the growth of contaminating fungal spores..

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Materials and Methods

Chemicals

Acetonitrile, Acetone , Ammonium hydroxide, Anhydrous Sodium Sulfate, Benzene, chloroform , glacial Acetic acid , hydrous disodium hydrogen phosphate (Na_2HPO_4 . $12H_2O$) . hydrochloric acid, methanol, potassium hydroxide, potassium hydroxide, potassium chloride, sulfuric acid, Sodium 1-hexana sulfonate sodium perchlorate, sodium chloride, and Tween 80 (supplied by BDH England monobasic potassium phosphate from fluka-swizerland Hexana supplied by Merch-w Germany Microcrystalline cellulse (Avicel PH 101, Avicel PH 301), Folic Acid, Maize Starch, Vitamin B1 (Thiamin mononitrite), Vitamin B2 (Riboflavin). Vitamin B6 (pyridoxine HCl). Methionine, talc and Magnesium Stearte supplied by FMC corporation and Kindly supplied from (Samara Drug Industries SDl. Iraq).

Microorganisms

Aspergillus flavus, Penicillia SPP. And Cladsporoia Cladosporoids were obtained from College of Agriculture, University of Baghdad. Cultures were stored on Sabouraud Agar slants following incubation at 25°C for five days Fresh cultures were prepared every four weeks.

Culture Media

Sabouraud Dextose Agar.
Rose Bengal Agar
macConkey Broth
solution used for dilutions and preparation of spore suspension.
Relative humidity of the prepared solution.
Extraction solvent of Aflatoxin.
Relative humidity Containers

Relative humidity of the prepared solution.

Table (1) represents the percentage of the relative humidity RH% of the prepared solutions as prescribed by AL Taher, 1990

Extraction solvent of Aflatoxin

According to howell and Taylor(1980) the extraction solvent of aflatoxin consists of acetonitrile : KCl 4% w/v: HCl 5N a ratio of 450 ml: 10ml.

Relative humidity Containers

Relative humidity containers consist of two glass containers connected one above the other and joined together by the cover of them and the cover is punched to allow the exchange of humidity between the two containers .

Table 1 : Relative Humidity of Various Solution

Substance	% of Substance in Solution	RH%
H_2SO_4	23%	75%
KCl	Saturated Solution	85%
$Na_2HPO_4.12H_2O$	Saturated Solution	95%

Assessment of Microbial Levels of the Raw Materials and active Ingredients

Sample of the following raw materials were collected from various sources and were subjected to microbiological assay according to the BP 1998 , as shown in table (2) in order to determine their microbial contents. Three types of raw materials were examined (Gelatin, Talc, and Starch) representing animal , mineral, and botanical origin, respectively As well as those of synthetic origin such as avicel, methionine, thiamin, pyridoxine, riboflavin and folic acid .

Samples were taken aseptically using the pour plate and memberane filtration techniques to determine the microbial level in the raw materials .

Table 2 : Isolation and Idetification Tests for Specified Microorganisms (BP , 1998)

Organism	Enrichment	Primary test	Secondary test	Confirmation
Enterobacteriaceae	Lactose broth 35-37 °C for 2-5 hr	EEB-Mossel 35-37 °C for 24-48 hr	VRBGLA 35-37 °C for 16-24 hr	Growth of Gram-negatives
E.coli	As above	MacConkey broth 43-45°C for 18-24 hr	MacConkey agar 43-45°C for 18-24 hr	Indole 43.5-44.5°C Biochemical
Salmonella	As above For 5-24 hr.	TBBG broth 42-43°C For 18-24 hr. Then subculture on: DCA,XLDA or BGA 35-37 °C for 24-48 hr.	TSI agar 35-37 °C for 18-24 hr.	Biochemical serological
Ps.aeruginosa	Saline peptone 35-37 °C for 2-5 hr	Casein digest broth 35-37 °C for 24-48 hr.	Cetrimide agar 35-37 °C for 24-48 hr.	Oxidase test
Staph.aureus	As for Ps.aeruginosa above	As for Ps.aeruginosa above	Baird-Parker 35-37 °C for 24-48 hr.	Coagulase. Catalase,DNase Test

EEB-m-Mossel Enterobacteriaceae enrichment broth - Mossel ;VRVGLA,violet red bile agar

Preparation of dried spore powder

A 0.1 ml aliquots of 7 days cultures of A. flavus, Cladospoa and pencillia were inoculated onto the surfaces of predried sabouraud dextrose ager plates, these were incubated at 25°C for 5 days. After this spores were clearly visible on all the plates. Three millimeters of stetrile water containing 0.1% Tween 80 (as a dispersing agent) were added to each plates. The spores were then dislodged by using glass spreader. The spore suspensions were obtained then stirred by using a vortex mixer for one minute. The spore suspensions were then filtered through a sterile cotton wool in order to get rid of the hypha. The filtrates were then harvested by centrifugation at (10,000xy) for 10 min) the superntanat liquids were then decanted and the residues were resuspended in 20 ml of sterile distilled water, washing were repeated three times . The number of spores of the resultant spore suspensisons was determined by aviable count technique, spore suspensions were adjusted so that the following suspensions were obtained, as 2.16x106 spore/ml for A flavus, 1.68x106 spore/ml forpenicillia spp. And 1.98x106 spore/ml for c.cladosporoids.

Tablet formulation

Multivitamin tablets and folic acid tablets were prepared using the formulas listed in tables (3) and (4), respectively.

The following excipients were used. Avicel PH 301 as a direct compression excipient, starch (5% w/w) as a disintegrant, magnesium stearate and stearic acid as lubricants. They were mixed with the active ingredient and compressed directly using a single punch tabletting machine with 7-mm flat-faced punches

Table 3 : The Formula of the Prepared Folic Acid Tablet.

Ingredient	Amount/tab.
Folic Acid	1 mg
Avicel PH 301	118.6 mg
Maize Starch	6.5 mg
Mg. Stearte	2.6 mg
Talc	1.3 mg
Total	130 mg

Table 4: The Formula of the Prepared Multivitamin Tablet

Ingredient	Amount / tab
Tianmin Mononitrite	1.5 mg
Riboflavin U.S.P.	2.0 mg
Pyridoxine HCl U.S.P	2.0 mg
Methionine	2.0 mg
Avicel PH 301	105 mg
Maize Starch	6.5 mg
Taic U.S.P	6 mg
Stearic Acid (Powdered)	3.0 mg
Mg.Stearte (Powdered)	2.0 mg
Total	130 mg

Preparation of contaminated tabltes:

For preparation of 500 contaminated tablets (0.3 ml. 30 ml) of A. flavus (0.4 ml 40ml) of penicillia spp and (0.3ml, 30ml) of C. cladosporoids were transferred to a sterile mortars and placed in the incubator until completely evaporation of water. Dried spores were scraped off and were included in direct compression formulations by dry mixing to get 10^2 spore /gram and 10^4 spore/gram for each of A. flavus pencicilia spp and C. cladosporoids respectively. Ingredients including dried microorganisms spores were weighed and lightly mixed in a glass morter by the method of geometric dilution technique for 20 minutes preliminary experiments had established that this method gives a uniform distribution of the microorganisms within the formuation screen in the lubricant (magnesium stearte or stearic acid) and mixed for an additional 5 minutes .

Quantities each of 130 mg were accurately weighed and poured into 7-mm diameter compresed between flat –Faced punces using a single punch tableting machine which was disinfected with 70% alcohol and the feed shoe was heat sterilized before use.

Determination of viable number of spores in the prepared tablets:

Viable number of spores in prepareted tablets was determined immediately after their production at different compression forces and after storage up to 8 weeks eight tablets (total wt.=1gm) were disintegrated in tryptic soy broth (9ml) according toBP 1998 using a flask shaker and suitable serial dilution in tryptic broth were prepred. One –ml sample of each dilution was poured in sterile petridish and then 15 ml of molten dextrose agar was added to the plate.

The sample and molten sabouraud dextrose agar were mixed together in forward and backward movement and swirled movement. The plate were allowed to solidify on surface . the plates were incubated at 35°C for 2-5 days. Survivals as colony forming units were estimated as the mean of triplicate determination and expressed as a percentage relative to an uncompressed control samples of the contaminated formulation.

Physical properties of tablets:

Thirty tablets were prepared using different compression pressure (137.9, 144.8 and 148.3MN/m^2) the physical properties of the tablets were determine (plumpton, 1982), these are tablet weight, thickness, friability, hardness (breaking strength), and disintegration time

Assay of Tablets:

An HPLC method was used for the assay of multivitamin tablets and folic acid tablets.

Assay for pyridoxine hydrochloride and thiamin multivitamin Tablets

The assay for pyridoxine hydrochloride and thiamin in multivitamin tablets was done according to the U.S.P XXIV method.

Effect of storage under different Relative Humidities upon the Survival of A. flavus, Penicilline, and Cladospori in folic acid and Multivitamin Tablets

Folic acid and multivitamin tablets containing Aspergillus.flavus Cladosporia ,and penicillia spores prepared at a compression of 148 MN/m2 and stored at 35°C under different relative humidity's (RH). The relative humidity were 75%, 85% and 95% within airtight containers. Survival of the spores within the tablets was assessed as the mean viability for each group at time 0 and after 1,2, 4,6 and 8 weeks. The total viable counts of the uninoculated (control) folic acid and multivitamin tablets were measured directly after preparation and after 4,8 weeks of storage at 35°C and 75% RH, 85% RHor 95% RH.

Aflatoxin assay

The amount of aflatoxin produced after storage the tablets (multivitamin and folic acid) at different relative humidities (75,85 and 95)% were assed at different time intervals using a modified method of Howel and Taylor (1981), the modification includes the use of twenty five grams of multivitamin and flic acid tablets stored at 4,6 and 8 weeks intervals.

Results and Discussion

Microbiological quality of some raw materials used in the production of tablet and tablet ingredients

Microbiological evaluation of the raw materials used in the production of tablets is presented in table (5), the result show that synthetic materials such as folic acid, magnesium stearate ,thiamin, riboflavin, pyridoxine, methionine and microcrystalline cellulose (avicel PH 301) had no microbial contaminants thus they meet the requirement of the B.P 98 which specify that atotal viable aerobic count bacteria should be equivalent to or less than 103 c.f u/gm and a total viable count for fungi equivalent to or to less than 102 c.f.u/gm is accepted. Microcrystalline cellulose PH 101 although it is a syntheyic raw material , it showed the presence of pseudoumonas aeruginosa (2x 10^2 cfu/gm) . samples taken from different parts of the microcrystalline PH 101 container showed apresence of pathogens in upper part of the container this is because microcrystalline (MCC) is a highly hygroscopic material [17] through its capillary action when it is exposed to air .Table (5) also shows that raw materials of natural origin(maize starch ,gelatin and talc) had relatively higher microbial levels which are ($7*10^2$,$9*10^2$ and 102 C.F.U/gm)for starch, gelatin and talc, respectively than that of the synthetic origin. This finding is similar to that of lbrahim Y.K.E 1991 which is due to the fact that materials of natural origin is rich in all the necessary requirement of growth needed by the microorganism. in addition, the results indicate that raw materials derived from animal and botanical origin had a higher microbial level than that of mineral origin. This is in agreement with the reported hypothesis of Bonomi and Negrriti,Baggerman and Kannegiter [22],[23],[3] .However, the microbial level obtained still below the B.P 1998 requirements.

Table 5 : Microbial Contamination Levels in Some Pharmaceutical Raw Materials for the Production of Folic Acid and Multivitamin Tablets

* C.F U/gm	Starch	Gelatin	Talc	Avicel PH 301	Avicel PH 101	Mag Steara.	Folic acid B_1,B_2,B_3 Methionine
Total Viable Aerobic Count	**7×10^2**	**9×10^2 bacillus**	**10^2**	**	**	****	****
Total Viable Count for Fungi	****	****	****	**	**	****	****
Enterobacteriaces	****	****	****	**	**	****	****
Escherchia coli	****	****	****	**	**	****	****
Staphylucocus aureus	****	****	****	**	**	****	****
Pseudomonas aerugenosa	****	****	****	**	**	****	****
Salomonella	****	****	****	**	**	****	****

Tablet Evaluation:

Folic acid and multivitamin tablets were prepared as previously mentioned (tables 3 and 4 respectively). The prepared folic acid and multivitamin tablets were evaluated physically, chemically and microbiologially. The results are shown in table 6.

Table 6 : Physical Chemical and Microbiological (Control) Evaluation of Folic acid and Multivitamin Tablet

Tablet Evaluation	Multivitamin	Folic acid
Weight (7 mm)	130 mg	130 mg
C. Pressure	148.3MN/m^2	148.33MN/m^2
Wt. Uniformity	0.9%	0.9%
Hardness (Kp)	6.6± 0.4	6.5± 0.6
Thickness(mm)	2.85	2.85
Friability %	0.2	0/2
DisintegrationTime Assay	2.3 min	2 min
B. Pyridoxine %	133.78%	
B_1 Thiamin %	148.2%	
Folic acid %		90%
Microbiological Quality		
One day after preparati		less than 10 *CFU/gm
After storage for 8 weeks at 35 °C , 75 % RH		less than 10 CFU/gm
After storage for 8 weeks at 35 °C , 85 % RH		less than 10 CFU/gm
After storage for 8 weeks at 35 °C , 95 % RH		less than 10 CFU/gm

Effect of relative humidity upon the survival of .A flavus in multivitamin and folic Acid tablets

Figure1,2 show the effect of storage under different relative humidities (95,85 and 75)% upon survival of Aflavus. spores in multivitamin and folic acid tablets. The results indicate that at a contamination level of 10^4 spore/gm as shown in figure(1) and storage at 75% R.H a decrease in survival over the eight weeks storage period to 13% whilest storage at 95% RH, caused an initial reduction in viability followed by a substantial increase to 86% at the end of 8 weeks in multivitamin tablets. Agermination, mycelia growth and sporulation occurred.The same behavior with folic tablets but with lower percentages. Visible fungal growth and sporulation were

apparent on the tablets after 6 weeks of storage. Further storage for 8 weeks caused a tablets to afragment. Storage at 85% R.H also showed visible fungal growth after 6 week. Viability of the organisms decreased during the first week of storage. This was accompanied by visible signs of mycelia growth. The decreased viability was probably due to the transition from dormant to mycelia state. Tablets containing hygroscopic materials are much more to physically, adsorb substantial amounts of water. Avicel has the ability to pick up moisture by capillary action and loosening of inter particulate hydrogen bonds on exposure to high humidities [8] .The water requirements for microorganisms varies depending on the organism (Blair,1988) as mentioned before. For different mould spores the minimum R.H required for germination varies from 70 to 98% and the optimum temperature for growth of moulds vary from (23-40) °C.For Aspergillus, 80% humidity aw=0.81[23] is essential for spore germination and the optimum temperature is (30-40) °C.Multivitamin tablets show higher growth than folic acid tablets within the storage time especially 95%, R.H this may be due to presence of more nutrients like vitamins (B1.B2 and B3)carbon source (starch) and amino acids (metithionine) or what is called nutrient availability. When nutrients are abundant, growth will be sustained but when only atrace nutrients are present, growth will be minimal. Fungi need various nutrients in order to meet their energy needs and to form macromolecules such as proteins and DNA. Since fungi cannot synthesize carbohydrate, so the substrate showed contain these compounds ; however, they can growth in a substrate rich in proteins without carbohydrates , e.g. cheese , by using amino acids as carbon source. Another important nutrient is nitrogen . All fungi can assimilate organic nitrogen compounds, depending on the species , certain vitamins must also be present in substrate, while the fungus itself synthesized others. The most important factors for growth are temperature , water activity and oxygen besides the presence of nutrients. Figure 2 shows the effect of storage upon the percent of survival using 10^2 spore/gm of A. flavus in multivitamin and folic acid tablets.The results indicate that storge at 75% and 85%R.H showed a decrease in number of spores to zero percent in eight weeks duration whilst storage at 95% showed increase in number of spores to 420 percent for the same time. The result also indicate that there is a significant different ($p<0.05$) between the three humidites as well as there are significant different between multivitamin and folic acid tablets and a significant difference between the weeks of storage. The overall effects of these three factors (humidity, time, and type of tablet-nutrients) is increase in number of spores/gm tablets with increase in humidity, time and type of tablet nutritents which are R.H 95%, eight weeks storage and more nutrient (multivitamin tablet).

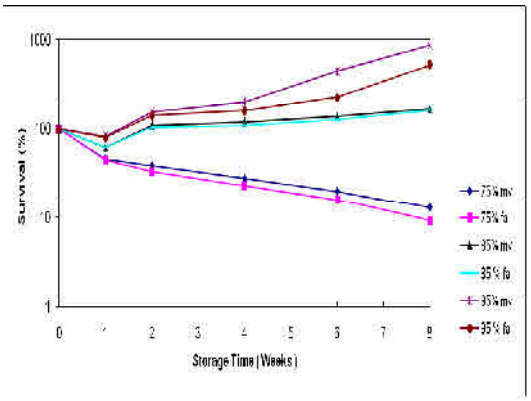

Figure 1: Effect of relative humidity upon survival of A. flavus (10^4) spore / gm compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.D$_{0.05}$ = 13.74 .

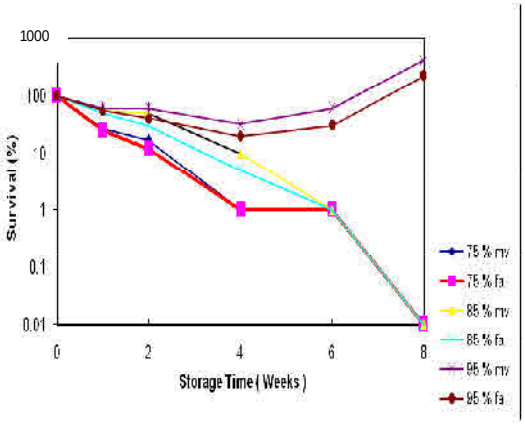

Figure 2 : Effect of relative humidity upon survival of A. flavus (10^2 spore / gm) compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.D$_{0.05}$ = 8.58 .

The overall effects of these three factors (humidity, time, and type of tablet-nutrients) is increase in number of spores/gm tablets with increase in humidity, time and type of tablet nutritents which are R.H 95%, eight weeks storage and more nutrient (multivitamin tablet).

The effect of R.H upon survival of Penicillia spp. Spores in multivitamin and folic acid Tablets:

Figures 3,4 show the effect of relative humidities upon survival of penicillia spp. Spores in multivitamin and folic acid tablets.

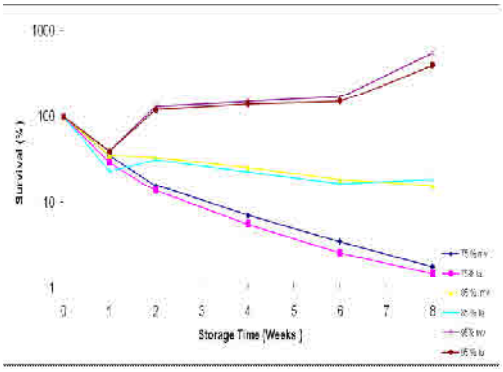

Figure 3 : Effect of relative humidity upon survival of Penicillia SPP. (10^4 spore / gm) compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.D$_{0.05}$ = 27.87.

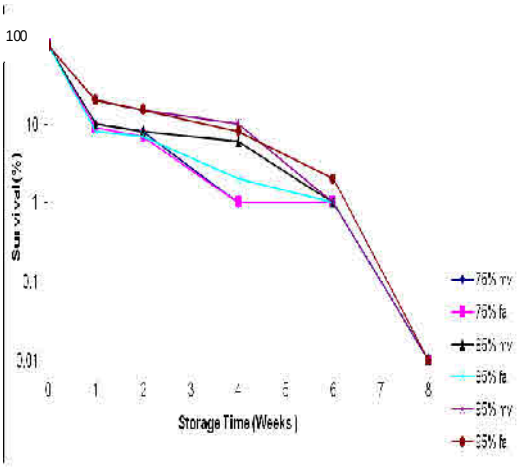

Figure 4 : Effect of relative humidity upon survival of Penicillia SPP. (10^2 spore / gm) compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.D$_{0.05}$ = N.S.

The results indicate that for both contamination levels of 10^4 and 10^2 spore/gm, storage at 75%RH, more decrease in survival of penicillia over the eight weeks storage period than A. flavus. was obtained i.e the decrease was to 1.8% and zero for 10^4 and 10^2 spore/gram, respectively. Whilst storage at 95% R.H however caused an initial reduction in viability followed by a substantial increase as germination mycelial growth and sporulation occurred for 10^4 spore/gram. Visible fungal growth and sporulation were apparent on the tablets after six weeks storage but the survival level was less than A. flavus. Further storage for eight weeks caused the tablets to fragment. On the other hand, storage at 85% R.H both contamination level 10^2 and 10^4 spore/gm, no visible fungal growth was apparent on the tablets after storage for eight weeks. Penicillia spp.. 80% humidity is essential for spore germination aw=0.84 [23] and the optimal temperature is (25-30) °C for most penicillia spp. The maximal temperature is (28-35) °C .the result also show that multivitamin tablets have more survival than folic acid for the three relative humidites used. So, the over all data indicate that there is a significant difference ($p<0.05$) between the three humilities, two types of tablets nutrients.As there is increase in anumber of spores/gm tablet with the increase in relative humidity, storage time, and type of tablet nutrients ..

The effect of storage under different relative humidities survival of Cladosporia cladosporoids Spores in multivitamin and Folic Acid tablets:

Figures 5,6 show the effect of storage at different relative humidities upon the percent survival of C. cladosporoids spores in multivitamin and folic acid tablets. The results indicate that there is a decrease in survival for both contamination level and storage at 75,85 and 95% R.H and for both types of tablets to zero percent. This is may be due to either temperature as cladosporia is psychrophilic mould (their low optimal temperature is 8-15) °C or water since 90-95% humidity is required for spore germinayion aw=0.88[23] this is not due to pressure because when cladosporia incubated at 25°C fungal spores were shown to retain viability over an eight weeks period (slight decrease in viability).In accordance with these water requirements tablets inoculated with A. flavus and Penicillia spores spoiled due to mould growth when stored under conditions (35°C , 95% R.H), while when stored under more moderate conditions (35°C 75% R.H) the tablets were not at risk to microbial spoilage both an optimum relative humidity and an optimum temperature are required before tablets are at risk of microbiological spoilage.

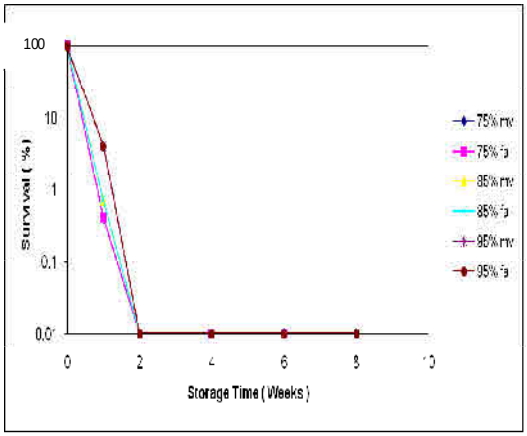

Figure 5 : Effect of relative humidity upon survival of Penicillia SPP C. Clado (10^4 spore / gm) compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.$D_{0.05}$ = N.S.

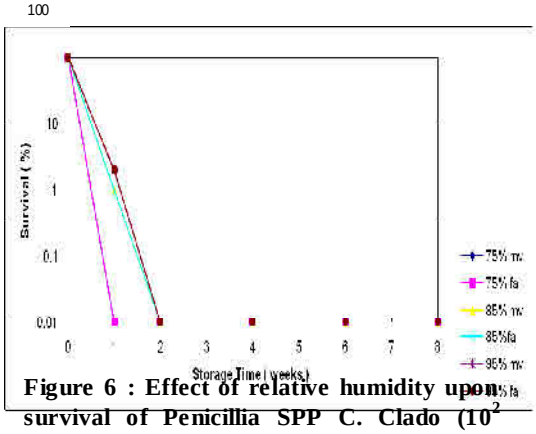

Figure 6 : Effect of relative humidity upon survival of Penicillia SPP C. Clado (10^2 spore / gm) compacted in multivitamin (mv) tablet and folic acid (fa) tablet at (148.3 MN/m^2) and stored at 35°C . L.S.$D_{0.05}$ = N.S.

The obtained results are in contrast to the results obtained by Fassihi and Parker [24] ,who found that tablets stored at a lower temperature (25°C) and at 96% R.H did not spoil due to mould growth (Aspergillus niger and penicillia spp) when stored under these condition and the viability of mould spores decreased slightly . On the other hand the results are in agreement with that of the study of Bos [19] in which a visible growth of A. Niger during storage at 100 and 95% R.H of sta-RX tablets and lactose/starch tablets stored at 25°C and 31°C respectively was obtained. If contaminants are introduced into tablets prior to processing (i.e. from the raw materials) then they might sitll eventually be responsible for the spoilage of the finished products . The nature of contaminating organism , the relative humidity at which tablets are stored and the tablet nutrient all contribute to survival of the organisms .

Aflatoxin Assay

Aflatoxin production is highly affected by type of substrate, the presence of minerals, the humidity and temperature [26 - 29] .Results were obtained from thin layer chromatography (TLC) and in camparison with standard showed that both multivitamin tablets and folic acid tablets contain no aflatoxin B1.If the environmental conditions (temperature, and relative humidity) are not suitable for fungal growth this will lead to decrease in aflatoxin production to a level that cannot be detected. Storage of tablets at 75% RH showed no aflatoxin B1 production, this result is in agreement with WHO [30] which determins that 83-85% RH is an optimum RH for AFB1 production by A. Flavus also this result is consistent with that obtained by Austwish[31] that determined 85% RH and more at temperature of 25°C is an optimum RH for A. flavus growth in addition, lakshinarasimham, and [32] determined that 20°C and RH 73.5% are considered as an optimum conditions for storage without fungal contamination. Furthermore storage at 85% RH showed no aflatoxin production, although RH is considerd as optimum for A. flavus growth but storage at 35°C is not optimum temperature for aflatoxin production since the optimum temperature for aflatoxin is (25-28)°C [33, 34] .Also, no aflatoxin production was noticed when storage at 95% RH although RH is considered as an optimum for A. flavus growth but 35°C is not an optimum temperature for aflatoxin production . Multivitamin tablet which contanins amino acid also show no aflatoxin production this because the storage temperature is not an optimum temperature for aflatoxin production so both an optimum temperature and relative humidity required for aflatoxin production .

Conclusions

The results showed the existence of relationship between type of the raw materials used in pharmaceutical production and its microbial level . The results showed the effect of various storage conditions upon survival, which depends upon the type of fungal spore, the hygroscopic nature of the excipient and the relative humidity of storage. Multivitamin tablet showed more survival than folic acid tablet and this is due to the presence of more nutrient. And finally no aflatoxin was obtained for both multivitamin and folic acid tablets at 35°C temperature, this is due temperature

which is not an optimum temperature for aflatoxin B_1 production.

Reference

1. Kalliugetal. Kallings, L.O: ringeretz o silverstorage : and emr feldt F. Microbiologgical contamination of medical preparations, Actapharma Suec1966: 3:219-228.
2. Sinugh p1 arirastaia B, kuma. A and dubey, N.K., Microb E.coli, , 2008 (56) : 555-560.
3. Kimiko farmati cheskii ehumnali, surivalal of microscopic fungi mnonsierile medical prrpation and auxillary subsiances , 2006: 40: 54-56
4. Obuexaeco, obekwe I.F. , ogbimi AO actapol. Pharm- drag res. , 2001: 53
5. Parker MT. Jowrnal of the Society of cosmetic chemists, 1978 : 23 :415
6. Fassihi, A.R., and parker, M.S inimicable effects of compaction speedon micro organisms in powder systems with dis_simiilar compaction mechanisms J.phase SCI , 1987 :76: 466-470
7. Plumpton EJ Gilbat,p, and fell JT the survival of micro organisms during tableting INT.J pharm, 1986a, 30 :241-146.
8. Blair, T.C, buckton, g, and bloomfiled, SF baird RJ hR. heak R.f and leachr (edj) microbial quality assurance pharmacent calts cosmeticals cosmetics and tioletric eis horwood chichest ppi 1988,104-116.
9. Blair , T.C graham bucton, sally F. bloomfield on the mechanism of kill of microbial of contanints during tablet compression int –jpharm ,1991:72: 111-115.
10. Fassihi A.R ,and palkar M.S the influence water activity and oxygentension upon the survival of aspergilly and penisillia species and tablet INT bio detior bull B, 1977:75-80
11. Waterman R.f sumner EFbldwn JN and warren F.W survival of stupply lococcus aureson pharmaceutical solid dosage form J. pharmsei , 1973 ,621 1317-1320.
12. Goda, n. k.s v. malath and R.uu suganthi effect of some chemical and herbal como under ongrowth of aspergillus parsiticesv and of latoxin production Animal feed science of technology , 2004,116:281-291.
13. abdullaMH1988 the isolation of aflatoxin from acacia and the incidence of Aspergillus flavus in the Sudan Mycopathologia, ; 1988, 104: 143-144.
14. Hitokoto, H 1978 H: muruzomi, s: Wauke, Tsakai, S: and Kuratah, Fungal contaminand mycotoxindetection of powdered herbal drugs Appl.
15. Wall heaver K,H micrological as aspects on the subject of oral solid dosage from pharm . Ind , : 1977 , 39 491-497.
16. AL- HiTi 1998 M.M.A Al janabi S. the effect of some peservits on preservative on Aspergilliaus flavus growth and its aflatoxin production Irqi J.pharm Scivol (10): 1998.
17. Aulton 2000 et., Me,: Tebby. H,G white p.J.p journal pharm, pharmacol, 26 suppl, 59p-60p 2000 Blair, T.C Graham Bucton; Sally F,: bloomfield on the mechanism of kill of microbial contaminants during tablet compression – Int –J- pharm , 1991. 72: 111- 115 .
18. Alfonoso 1985
19. Bos 89, C,E van doorne ,H lerk C.F. on microbiological stability of tablets stored under tropical conditions, : 1989 , 55: 175-183 .
20. Ibrahim Y.K.E, 1991Y.K.E; orinolou- pdf. Comparative microboial levels in levels in wet granulation and direct compression method of tablet production, ACTA HELV. : 1991 66: 11.
21. Bonomi, E. and nergetti, F. 1977 Bonomi E. and Nergetti, F. studies on the microbial content of raw materials used in pharmaceutical preparation Ann. 1st . super Sonita: 1977, 13:802-832.
22. Panlo. J Brasillianj of microbiology , 2006 ,vol. 37.no 1.
23. Northolt MD and bull ermine prevention of moldgrothed and Bullerman prevention of moldgroth and tex production through control of environmental condition .J. too production , 1982 ,45: 519- 526.
24. Fassihi and parker 1977 fassihi A.R and parker M.S the of water activity and oxygen tension upon the survival of aspergillus and penicllia tablet INT Biodeterior Bull.1977 , 13-80
25. Plumpton 1982 E.J studies upon the survival of various microorganism in solid dosage froms Ph.D. thesis university of Manchester. 1982
26. Reddy S.V M.D Kiram R.M. uma, K. thir umaladai and D.V.R ready aflatoxin B different grades of chillies, 2001, 18: 553-558,
27. Elad. D, .risk uma, assessment grades of malicious bio contamination of food tournal food protection, 2005, 68:1302-1305.
28. Shar pirar.o. control of mycotoxin storage and technigagus for their decontaminal my cotoxin in food detection and control.

Wood head publishing Cambridge u.K-

29. Northolt M.D. the effect water activity and temperature the production of some my cotoxines Diss wageninger 1979

30. WHO, 2003. Laboratory manual 2nd ediction interim guidelines WHO.

31. Austwish , p.k.c; and ayerst, G.groundaut micrflora and toxicily –chem. Ind 1963 ,55 -61.

32. Mukher 95 :and lakshminarasimiham A.B aflatoxin contamination of storage under 2004, 190-223.
controlled conditions int J med Microbiol Virol parassitol infect dis ., 1995 , 282(3): 387-43.

33. Bennet Jw, klichm my cotoxins- chinical microbiology review ; 2003,16:497-516.

34. Paterson rrm; venancio A; liman solution to penicillium taxonomy crucy to my eotoxin rerearch and heath research in microbiology ; 2004 ,155: 507-513.