International Journal of Research in Engineering, Science and Management
Volume 4, Issue 8, August 2021
https://www.ijresm.com | ISSN (Online): 2581-5792


# A Study on the Growth of *Lichthemia corymbifera* on Different Cultural Media which Demonstrate the Needs of Strong Antifungal Agent in Baird Parker Medium for the Isolation of *Staphylococcus aureus* from Spice Samples

Rijo Johny[1*], C. M. Srilatha[2], Dinesh Singh Bisht[3], A. Ranjith[4], R. Darsana[5]
[1]*Scientist-B, Spices Board, Navi Mumbai, India*
[2]*Scientist-C, Spices Board, Cochin, India*
[3]*Scientist-C, Spices Board, Navi Mumbai, India*
[4]*Scientist-C, Spices Board, Chennai, India*
[5]*Microbiologist, Spices Board, Cochin, India*

***Abstract*: A heavy fungal growth was observed on Baird Parker agar (BPA) plates while testing a chili ground sample for *Staphylococus aureus* as per USFDA BAM method and the fungus was identified as *Lichtheimia corymbifera.* The ability of *L. corymbifera* to grow in different bacterial media being used for spice sample analysis was assessed with two general and five selective bacterial media and compared with two fungal media. Upon 48 hours of incubation at 35± 1 ºC, the growth was calculated by measuring the diameter of fungal colony. BPA plates showed highest growth of 82±1mm in diameter followed by Nutrient Agar (NA) (65±1mm), Bismuth Sulfite Agar (BSA) (56±1mm), Potato Dextrose Agar (PDA) (52±1mm), Plate Count Agar (PCA) (50±1mm), Dichloran Glycerol Agar (DGA) (41±2mm) and Violet Red Bile Glucose Agar plates (VRBGA) (30±1mm). Measurable growth of *L. corymbifera* was not observed in Xylose Lysine deoxy-cholate Agar (XLDA) and Hecktoen Enteric Agar (HEA) plates for 48 hours of incubation at 35 ºC. The results indicated that the bacterial media such as BPA, NA, BSA, PCA and VRBGA were not able to inhibit the growth of *L. corymbifera* to a desirable level. The present study emphasizes the need to incorporate extra antifungal agents in BPA medium whenever used for spice sample analysis.**

***Keywords*: Bacterial media, BPA, *Lichtheimia corymbifera*, antifungal agent, spices.**

## 1. Introduction

A suitable culture media is one of the prerequisites for isolation, identification and maintenance of microorganisms. Various factors are being considered while formulating a culture media for bacteria and fungi. The fungal growth is less common in bacterial medium due to factors such as short incubation time, neutral pH, composition of medium, higher incubation temperature etc. [1], [2]. Optimum temperature for the growth of most fungi and bacteria is 25 ºC (22 to 30 ºC) and 35 to 37 ºC respectively. [3] Bacteria prefer a neutral pH [1.3] whereas most fungi prefer an acidic pH for their growth. [4] Since fungi require lesser water activity ($a_{w=}$0.70) for growth than bacteria ($a_w$=0.90), the water activity of bacterial medium is not a limiting factor for fungal growth. [3], [4] Media with high contents of carbohydrate, nitrogen and minerals at a pH range of 5 to 6 [5] are required for the growth of fungi. The incubation period for most bacteria lies between 1 to 2 days whereas it requires 3 to 5 days for a fungus. The doubling or generation time of most bacteria is considerably lesser than fungi, hence bacteria develop colonies faster than fungi on the growth media. These features of bacteria and fungi are being utilized in the formulation of bacterial and fungal media.

The fungus used in the present study, *Lichtheimia corymbifera* was earlier known as *Absidia corymbifera. L. corymbifera* which belongs to the class Zygomycetes and family Mucoraceae. It is found worldwide in soil, decaying organic matter, damp building materials [6] and is linked to many adverse health effects**.** [7] *Lichtheimia* species are also found in spices [8], nuts [9] and grain products. A study found that *Lichtheimia* spp are present in 16 % of poultry feed mixtures [10]. Qaher Mandel in 2005[11] reported 0.6% occurrence of *L.corymbifera* in spices.

*L. corymbifera* is psychrotolerant and thermophilic fungus with optimum growth temperature of 37°C. Its' maximum growth temperature is as high as 48 to 52°C and it can also grow at a pH range of 3.0 to 8.0. [12] The minimum water activity required for the spore germination and growth in vitro for *L. corymbifera* is 0.88.[13] The ability of *L. corymbifera* to grow in higher temperature in a rapid manner coupled with its

*Corresponding author: rijo83@gmail.com

presence in spices may result in the contamination of media meant for bacterial enumeration. This aspect is studied in this paper. Since most of fungi are not capable to grow in bacterial media in a short incubation time of 48hrs, the probability of contamination by fungi during selective isolation and identification of bacteria has not given much importance.

Most of the general and selective bacterial media do not have antifungal agents included in their composition. The objective of the present study was to highlight the need of incorporating compatible antifungal agent in selective bacterial media like BPA used for spice sample analysis in order to inhibit the contaminating fungi and recover the specific bacteria.

## 2. Materials and Methods

### *A. Isolation and identification of Lichtheimia corymbifera*

The fungal growth observed on BPA as a contaminant while performing *S. aureus* analysis of chili samples was isolated on PDA plates. Cultural characteristics were observed and simple staining was done using crystal violet for observing the morphology. Further, the culture was sent to National Fungal Culture Collection of India (NFCCI), Pune for identification. The fungus was identified as *Lichtheimia corymbifera* based on morphological characters.

### *B. Media*

A comparative growth study of *L. corymbifera* was performed in nine different media. The media taken here are routinely used for the microbiological analysis of food products as per USFDA BAM. The details of the media used for the study as follows,

*1) Baird Parker Agar Base (BPA)*

BPA contains 10g of casein enzyme hydrolysate, 5g of beef extract, 1g of yeast extract, 12g of glycine, 10g of sodium pyruvate, 5g of lithium chloride and 20g of agar. The media was prepared by adding above ingredients into 950ml distilled water, heated to dissolve medium completely, sterilized and cooled to 50°C and aseptically added 50ml of egg yolk tellurite emulsion. The final pH was adjusted to 7.0±0.2.

*2) Bismuth Sulfite Agar (BSA)*

BSA contains 10g of peptic digest of animal tissue, 6g of beef extract, 0.4g of ferric citrate, 0.01g of brilliant green, 3g of bismuth ammonium citrate, 10g of sodium sulfate, 5g di-sodium hydrogen phosphate, 5g of D-glucose anhydrous and 24g of agar. The media was prepared by adding the above ingredients into 1000ml of sterile distilled water, heated to dissolve medium completely and boiled for 01min. The final pH was adjusted to 7.6±0.2.

*3) Dichloran Glycerol Medium (DGA)*

DGA contains 5g of peptic digest of animal tissue, 10g of dextrose, 1g of monopotassium phosphate, 1g of monopotassium phosphate, 0.002g of magnesium sulphate, 0.10g of chloramphenicol, 15g of agar. The media was prepared by adding the above ingredients to 500ml distilled water, heated to dissolve medium completely, 110g of glycerol was added and sterilized. The final pH was adjusted to 5.6±0.2.

*4) Hektoen Enteric Agar (HEA)*

HEA contains 12g of peptone, 3g of yeast extract,12g of lactose, 12g of sucrose, 2g of salicin, 9g of bile salt mixture, 5g of sodium chloride, 5g of sodium thiosulphate, 1.5g of ferric ammonium citrate, 0.1g of acid fuchsin, 0.065g of bromothymol blue-, 14g of agar. The media was prepared by adding the above ingredients to 1000ml of sterile distilled water and heated to dissolve medium completely. The final pH was adjusted to 7.5±0.2C.

*5) Nutrient Agar (NA)*

NA contains 10g of peptic digest of lean meat, 10g of beef extract, 5g of sodium chloride and 20g of agar. The media was prepared by adding the above ingredients to 1000ml distilled water, heated to dissolve medium completely and sterilized. The final pH was adjusted to 7.6±0.2.

*6) Plate Count Agar (PCA)*

PCA contains 5g of casein enzyme hydrolysate, 2.5g of yeast extract, 1g of dextrose and 15g of agar. The media was prepared by adding the above ingredients to 1000ml distilled water, heated to dissolve media completely and sterilized. The final pH was adjusted to 7.0±0.2.

*7) Potato Dextrose Agar (PDA)*

PDA contains 4g of potato infusion, 20g of dextrose and 20g of agar. The media was prepared by adding the above ingredients to 1000ml distilled water and sterilized. The final pH was adjusted to 5.1±0.2.

*8) Violet Red Bile Glucose Agar (VRBGA)*

VRBGA contains 7g of Pancreatic digest of gelatin, 3g of Yeast extract, 5g of Sodium chloride, 1.5g of Bile salts, 10g of D(+) Glucose monohydrate, 0.03g of Neutral red, 0.002g of Crystal violet, 15g of Agar. The media was prepared by adding the above ingredients to 1000ml of sterile distilled water and heated to dissolve medium completely. The final pH was adjusted to 7.4±0.2.

*9) Xylose Lysine Deoxy-cholate Agar (XLDA)*

XLDA contains 3.5g of xylose, 5g of L-lysine, 7.5g of lactose monohydrate, 7.5g of sucrose, 5g of sodium chloride, 3g of yeast extract, 0.08g of phenol red, 2.5g of sodium deoxycholate, 6.8g of sodium thiosulfate, 0.8g of ferric ammonium citrate and 13.5g of agar. The media was prepared by adding the above ingredients to 1000ml of sterile distilled water and heated to dissolve medium completely. The final pH was adjusted to 7.2-7.6.

*10) Evaluation of L. corymbifera growth in different media*

*L. corymbifera* was inoculated into the chosen nine media. The plates were incubated at 35ºC± 1 ºC upto 48 hours. Three replicate plates were maintained for each culture media. Concurrent control plates were also maintained. The fungal growth in the media plates were measured at every eight hours' interval by using a calibrated scale. Diameter of the fungal colony was used as a measure of growth [4].

## 3. Result and Discussion

The average diameter of *L. corymbifera* growth observed in nine different media at an interval of 8hrs for 48hrs and their standard deviation are given in Table 1. The BPA medium showed highest growth; 82±1mm in diameter followed by NA, BSA, PDA, PCA, DGA and VRBGA which showed 65±1mm, 56±1mm, 52±1mm, 50±1mm, 41±2mm and 30±1mm

respectively. The photographs of growth of *L.corymbifera* on different media is shown in figure1. Measurable fungal growth was not observed in the XLD and HEA plates. The graphical representation of above results is given in figure 2.

Table 1
Growth of *L.corymbifera* on different media at 35± 1 ºC for 48hrs:
The values represent mean ± SD (n =6)

| Growth media | Diameter of *L.corymbifera* colonies in mm | | | | | |
|---|---|---|---|---|---|---|
| | 8hrs | 16hrs | 24hrs | 32hrs | 40hrs | 48hrs |
| BPA | 15±1 | 30±1 | 44±2 | 66±1 | 74±2 | 82±1 |
| PDA | 8±1 | 14±1 | 24±1 | 33±1 | 40±1 | 52±1 |
| DGA | 7±1 | 11±1 | 20±1 | 27±1 | 33±1 | 41±2 |
| PCA | 7±1 | 17±1 | 22±1 | 33±1 | 41±1 | 50±1 |
| NA | 9±1 | 23±1 | 37±1 | 52±1 | 54±1 | 65±1 |
| BSA | 8±1 | 16±1 | 30±1 | 42±1 | 48±1 | 56±1 |
| XLDA | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| HEA | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| VRBGA | 5±1 | 9±1 | 15±1 | 21±1 | 25±1 | 30±1 |

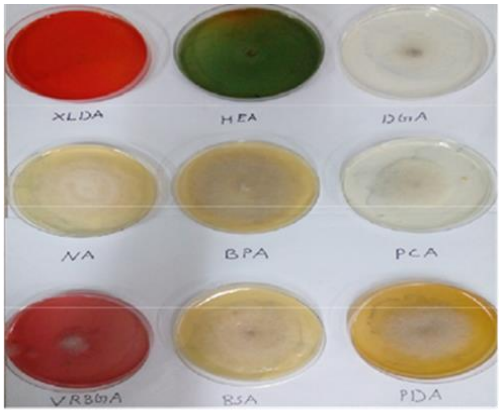
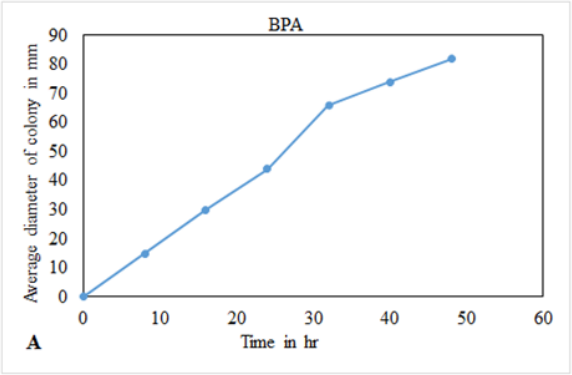
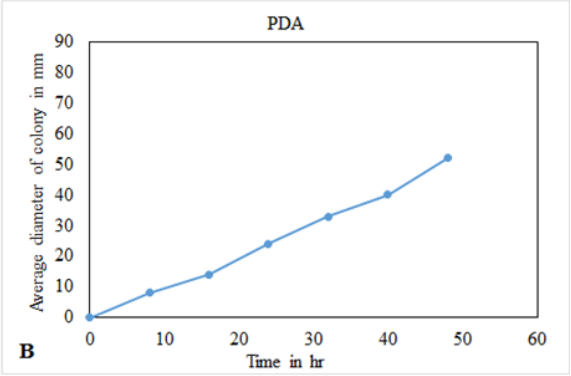
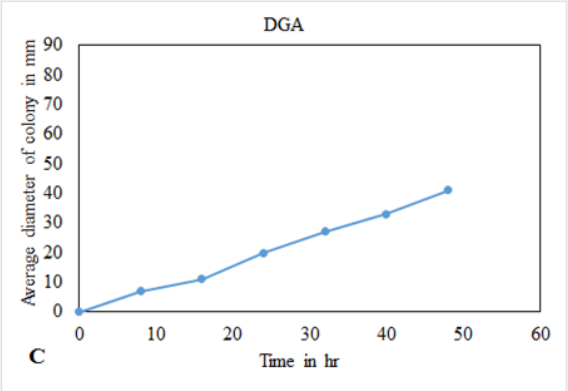
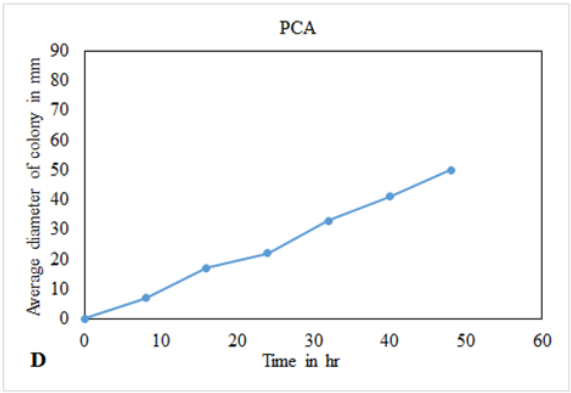


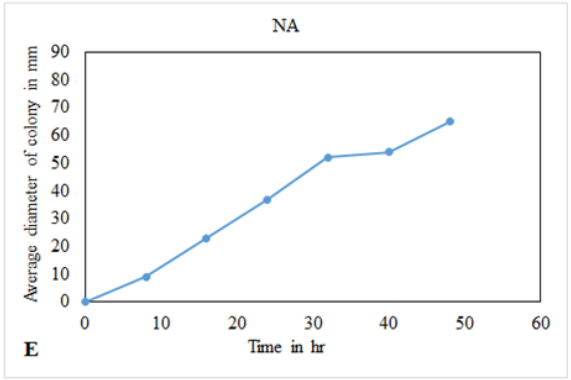

Fig. 1. Photograph of growth of shows the growth of *L.corymbifera* on different media after 48hrs of incubation

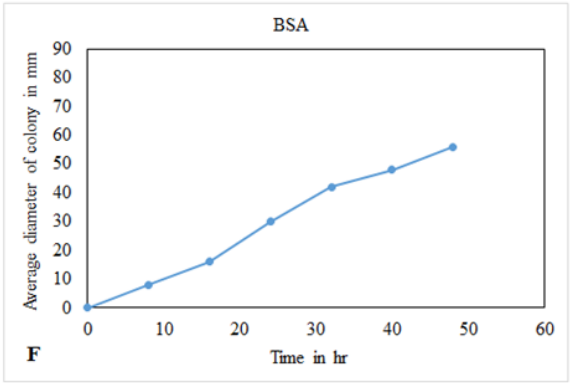


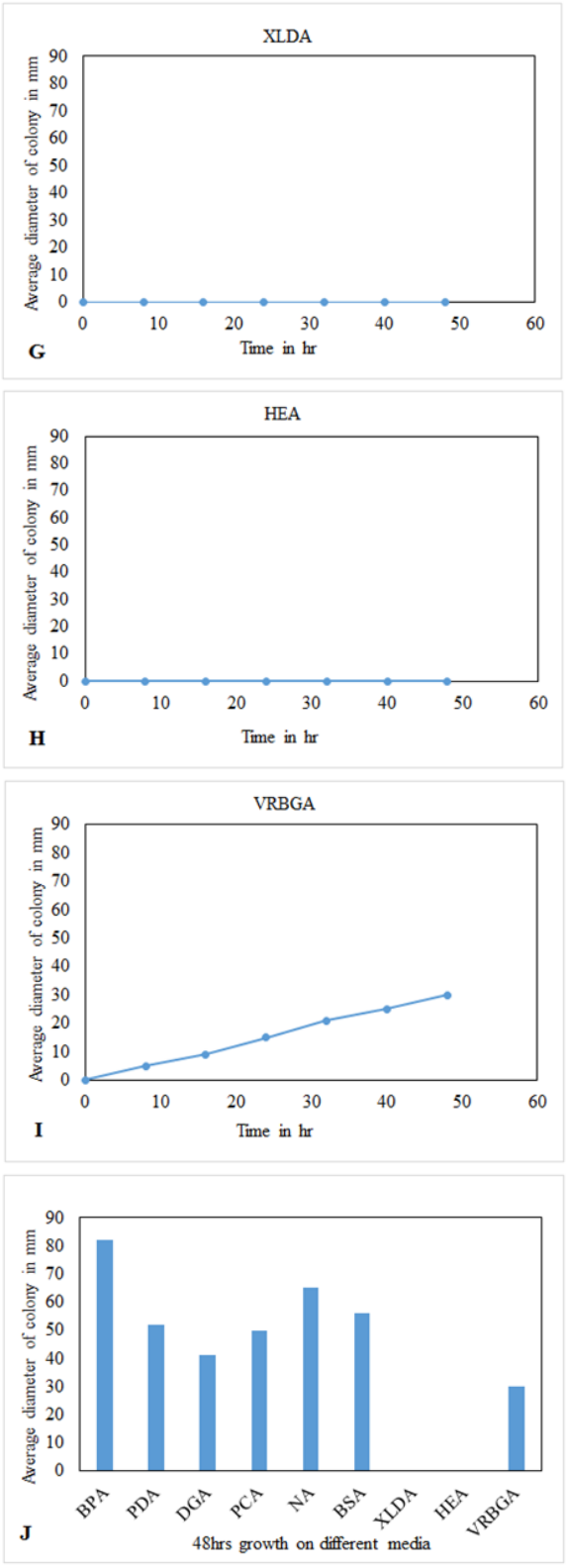



Fig. 2. Graphs A, B, C, D, E, F, G, H and I shows the growth of *L.corymbifera* at different time intervals on different media like BPA,PDA,DGA,PCA,NA,BSA,XLDA,HEA and VRBGA. Graph J shows the growth of *L.corymbifera* on different cultural media after 48hrs of incubation

Baird Parker Agar, developed by Baird Parker [14], [15] from the Tellurite-glycine formulation of Zebovitz et al. [16] has been used for isolation and enumeration of *Staphylococci* in food and other material since it allows a good differentiation of coagulase positive strains. Lithium chloride and potassium tellurite present in the medium inhibits most of the contaminating microflora except *Staphylococcus aureus*. [14,15] Lithium chloride is having narrow antifungal spectra which will limit development of specific fungi on isolation plates. Lithium chloride is not having inhibitory effect to most of the fungi belongs to class Zygomycetes. [17] L. corymbifera which belongs to the class Zygomycetes and family Mucoraceae. *L. corymbifera* has shown approximately 82mm growth in BPA in 48hrs of incubation at 35 ºC that is the same incubation temperature and time suggested by USFDA BAM method for the isolation of *S. aureus* in BPA medium. The single colony of *L. corymbifera* covered 91% of the petri dish in 48hrs of incubation that makes isolation and identification of *S. aureus* difficult in BPA medium. The BPA is highly nutritious due to presence of enzymatic digest of casein and beef extract as the carbon and nitrogen sources, Yeast Extract as B-complex vitamins [14,15], Glycine, Sodium Pyruvate and egg yolk also make this medium more nutritious for this fungus. The Lithium Chloride and 1% Potassium Tellurite Solution in BPA could not inhibit the growth of *L. corymbifera*.

The similar or higher *L. corymbifera* growth was also observed on BSA, NA and PCA plates than the fungal media like PDA and DGA. The BSA medium is having Peptic digest of animal tissue and beef extract which serves as sources of carbon, nitrogen, vitamins and essential growth factors. Dextrose is the carbon source and disodium phosphate maintains the osmotic equilibrium. [18] Bismuth sulphite indicator along with brilliant green could not inhibit the growth of *L. corymbifera*. NA and PCA are general bacterial medium which contain carbon and nitrogen sources sufficient to support the growth of *L. corymbifera*. Among the media which supported the growth of *L. corymbifera*, VRBGA shown the least growth. This might be due to the presence of any one of the following chemical in the VRBGA like bile salts mixture, neutral red or crystal violet.

The media like XLDA and HEA inhibited the growth of *L. corymbifera.* Both media are selective bacterial medium which contain both nutrients and selective chemical agents. The presence of Deoxy-cholate, ferric ammonium citrate and sodium thiosulphate in XLDA might have inhibited the growth of the fungus. Bile salts, bromthymol blue and acid fuchsin in HEA that might have inhibited the growth of *L. corymbifera.* This experiment shows the need of a thorough investigation to find a suitable chemical which can suppress the growth of fungus and in same time, it should support the growth of bacteria of interest.

## 4. Conclusion

The fungus, *L. corymbifera* used here was obtained as a contaminant from BPA while performing *S.aureus* analysis of chili sample by USFDA BAM method. *L. corymbifera* is found worldwide in soil and decaying organic matter and is linked to many adverse health effects. Due to its occurrence in food products especially spices, nuts etc., the fast growing nature and optimum growth temperature and pH similar to most of

bacteria, it can be a contaminant in bacterial culture media. Such fungal growth in a bacterial medium can suppress the growth of the concerned bacteria of interest.

Though there are chances of other bacterial or fungal growth in selective media, in the current study, the fungus *L. corymbifera* was grown rapidly within a short incubation period of 48 hours displacing the bacteria of specific interest, *Staphylococcus aureus* thereby making the specific bacterial isolation and identification difficult. The fungal growth in BPA indicates that the selective media does not have the required media components in it to inhibit the fungal growth. The media chosen for the study such as BPA and BSA are routinely used in microbiological analysis of spice samples as per USFDA BAM. Keeping into consideration the importance of the said media, a thorough examination is required for the incorporation of compatible antifungal agents that shall not interfere with the growth of the bacteria of interest. Thus the above study necessitates the need of extra antifungal agents in BPA.

## Acknowledgement

The authors are thankful to Spices Board India for providing the facilities and National Fungal Culture Collection of India (NFCCI) for the identification of the fungus.

## References

[1] Rosso L., Lobry J. R., Bajard S., Flandrois J. P. 1995. Convenient Model to Describe the Combined Effects of Temperature and pH on Microbial Growth. *Applied and environmental microbiology*, 61(2): 610-616.

[2] Rousk J., Brookes P.C., Baath E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralisation. *Applied and Environmental Microbiology*. 75(6):1589-1596.

[3] Mcmeekin T.A., Chandler R.E., Doe P.E., Gariland C.D., Olley J., Putro S, Ratkowsky D.A. 1987. Model for the combined effect of temperature and salt concentration/water activity on growth rate of Staphylococcus xylosus. *Journal of Applied Bacteriology*. 62(6):543-50.

[4] Gibson A.M., Baranyi J., Pitt J.I., Eyles M.J., Roberts T.A.1994. Predicting fungal growth: effect of water activity on *Aspergillus flavus* and related species. Int. *J. Food Microbiol*. 23(3-4): 419–4310.

[5] Calile M. J., Watkinson S. C., Gooday G. W. 2005. *Fungi* (Second edition). Elsevier, New Delhi.

[6] Samson R.A., Hoekstra E.S., Frisvad J.C. 2004. Introduction to food and airbone fungi. 7th, -389 p. Baarn, Centralalbureau voor Schimmell cultures, Institute of the Royal Netherlands Academy of Arts and Sciences.

[7] Garcia-Hermoso D., Hoinard D., Gantier J.C., Grenouillet F., Dromer F., Dannaoui E. 2009. Molecular and Phenotypic Evaluation of *Lichtheimia corymbifera* (Formerly *Absidia corymbifera*) Complex Isolates Associated with Human Mucormycosis: Rehabilitation of L. ramosa. *Journal of Clinical Microbiology*, 47 (12): 3862–3870.

[8] Gatti M. J., Fraga M. E., Magnoli C., Dalcero A. M., Darocharosa, C. A. 2003. Mycological survey for potential aflatoxin and ochratoxin producers and their toxicological properties in harvested Brazilian black pepper. *Food Addit.Contam.* 20(12): 1120-1126.

[9] Mphande F. A., Siame B. A., Taylor J. E. 2004. Fungi, aflatoxins, and cyclopiazonic acid associated with peanut retailing in Botswana. *J Food Prot*. 67(1): 96-102.

[10] Labuda R., Tancinova D. 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. Annals of Agricultural and Environmental Medicine. 13(2): 193-200.

[11] Qaher A. M. (2005). Fungal contamination of some imported spices. *Mycopathologia*. 159: 291-298.

[12] ST-Germain G., Summerbell R. 1996. Champignons filamenteux d'intérêt médical. Caractéristiques et idenfication, 314 p. Belmont, Star Publishing Company.

[13] Hocking A. D., Miscamble B. F. 1995.Water relations of some Zygomycetes isolated from food. *Mycological Research*. 99(9): 1113-1118.

[14] Baird-Parker A. C. 1962. An improved diagnostic and selective medium for isolating coagulase positive S*taphylococci*. *Journal of Applied Bacteriology* 25(1): 12-19.

[15] Baird-Parker A. C., Davenporte 1965. The effect of recovery medium on the isolation of *Staphylococcus aureus* after heat treatment and after storage of frozen or dried cells. *Journal of Applied Bacteriology*, 28(3):390-402.

[16] Zebovitz E., Evans J. B., Niven C.F. 1955. Tellurite-glycine agar: a selective plating medium for the quantitative detection of coagulase positive *Staphylococci*. J. Bacteriol. 70:686-690.

[17] Dana L Richter 1, Sara C Robinson, Maria P Beardslee, Maureen L Habarth 2008.Differential sensitivity of fungi to lithium chloride in culture media. Mycol Res. 112(Pt 6):717-24.

[18] Tinall B. J., Crimont P. A. D., Gorrity G. M., Euzesy B. P. 2005.Nomenclature and taxonomy of the genus Salmonella. *International Journal of Systematic and Evolutionary Microbiology*, 55(1): 521-524.