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Research Article

Studies of various microbial contaminants during culturing of amniotic fluid derived cells in buffalo (*Bubalus bubalis*)

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Abstract

Cell culture complexity is as shared as that of culture failure due to contamination. All cell culture in laboratories and cell culture workers have acknowledged it. Culture contaminants may be organic or elementary, seen or hidden, unhelpful or apparently gentle, but in all gear they harmfully influence both the use of cell cultures and the excellence of research work. Major microbial contaminants in the cell culture include Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Escherichia. coli, Mycoplasma orale, M. fermentans and Saccharomyces cerevisiae. There are several factors which influence the amniotic fluid (AF) cell culture like level of blood cells, volume of amniotic fluid, attachment time, number and microbial contamination. Amniotic fluid derived cells in buffalo (Bubalus bubalis) were cultured in normal suitable media and effects of microbial contaminants were studied.

Keywords: *Amniotic fluid cell, Microorganism, Buffalo, Cell culture*

1. Introduction

The majority noticeable result of cell culture contamination is the failure of time, money and attempt tired rising cultures and location up experiments. However, the fewer obvious cost is often more serious. First there are unfavorable special effects on cultures suffering from hidden chemical or organic (biological) contaminants. These unseen contaminants can attain elevated densities changing the development and individuality of the cultures. Worse, yet are the potentially incorrect or mistaken results obtained by innocently working with these cryptically impure cultures. Harvest, such as vaccines, drugs or monoclonal antibodies, manufactured by these cultures will possibly be ineffective. For a number of researchers, the harshest outcome of contamination is suffering the discomfiture and damage to their status that results when they inform collaborators or journals that their investigational results are defective and have to be retracted due to contaminants in their cultures.

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Preventing all cell culture contamination has long been the dream of many researchers, but it is an impractical, if not impossible, dream.

It has been reported that AF could be a promising source of putative stem cells, and that the AF cells are easily reprogrammable than other somatic cells (Li *et al*., 2009; Galende *et al*., 2010). Ethical problems and material sourcing, which limit embryonic stem cell research lead to isolation of cells from human amniotic fluid and have been named them human amniotic fluid stem (AFS) cells (Ryan ,1998) thereby providing a new route for stem cell research (Chiavegato *et al*., 2007). The information on AFS cells in buffalo species is very rare and has been reported from a few labs only (Yadav *et al*., 2011; Dev *et al*., 2012a, b). In the present investigation, effect of various microbial contaminants that influence the primary AF cell culture have been studied.

2. Materials and methods

2.1 Chemicals and media

All chemicals, reagents, culture media and antibiotics used during the study were of cell culture grade, obtained from Hi Media Laboratories (Mumbai, India)

unless otherwise indicated. Fetal bovine serum (FBS) was from Hyclone (Thermo Scientific, USA) and Trizol was from Invitrogen (USA). Disposable 35mm x10mm cell culture Petri dishes, 6 well tissue culture plates and centrifuge tubes were procured from Tarsons Products Pvt. Ltd. (Kolkata, India). Membrane filters were from Advanced Micro-devices (Ambala, India). The primers were got synthesized from GenxBio (India). The culture media were reconstituted freshly as per manufacturers' instructions and filter-sterilized (0.22µm) prior to use.

3. Transportation and collection of sample

Buffalo gravid uteri were obtained from a nearby abattoir, washed 2-3 times with isotonic saline fortified with 1% of penicillin/streptomycin and transported to the laboratory in a thermally insulated ice box within 5 hour. Uterine cut, fetus and membranes were located and AF was aspirated aseptically with the help of 20 ml syringe fitted with 16 gauge hypodermic needle. Twenty millilitres of AF was collected in Oakridge tubes. The appearance (fluid without cells, blood-cell-free, blood-cell, bloody or brown-coloured fluid), volume of fluid collected were observed and carefully documented.

3.1 Culturing of AF cells

AF cells were isolated from buffalo amniotic fluid after being centrifuged at 3000 rpm for 10 min and cell pellet was washed three times with Dulbecco's phosphate-buffered saline (PBS) containing 1% antibiotics (penicillin/streptomycin) followed by seeded at density of 10^3 cells/ cm^2 in 6 well culture plates containing cell culture medium (DMEM supplemented with 16% FBS, 1% L-Glutamine, 1% penicillin/streptomycin) and incubated in humidified CO_2 incubator (Lark, China) at 38.5±0.5˚C in presence of 5% CO_2 in air (Dev *et al*., 2012a,b). The cells were allowed to grow and sub cultured by passaging after achieving 80-90% of confluency without the use of feeder layer. Viability of the cells was determined by trypan blue dye method and cells were counted with a hemocytometer (ROHIM, India). We monitor the effect of microorganisms in the cell culture medium like change in pH value, change in growth kinetics and viability of the cell's day by day.

3.2 Observation of microbial contaminants during AF cell culturing

After the isolation and culturing of AF cells from buffalo amniotic fluid, microbial (bacterial, fungal and mycoplasm) contaminants were found virtually everywhere and were able to quickly colonize and flourish in the rich and relatively undefended environment provided by cell cultures. Because of their size and fast growth rates, these microbes are the most commonly encountered cell culture contaminants (Fig. 1-3).

3.3 Effect of microbial contaminants on pH of the culture medium

pH of the culture medium reduced from normal alkaline to low acidic during the incubation periods. The pH of the normal culture medium (control) was observed as 7, 7, 7.2, 7.4, 7.5, 7.7, 7.8, 8.1 and 8.3 from first to nine days of incubational periods respectively. But in case of the contaminated culture, pH decreased from 7, 6.9, 6.6, 6.2, 5.9, 5.7, 5.4 to 5 from first to nine days of incubational periods respectively (graph 1).

3.4 Effect of microbial contaminants on growth kinetics and viability of culture medium

Microbial contaminants affect the growth kinetics and viability of cultured cells. In healthy medium, cells almost double in five day time period of incubation (graph 2). In case of the contaminated culture medium, cell number reduced rapidly nearly to half as from when the cell viability was studied *vis a vis* contamination. It was found that in contamination free culture, cell viability was 86% as compared to the contaminated one where it was 32 % at 7^{th} day of culture (graph 3).

4. Results and Discussion

Every day inspection of cultures will make sure premature finding of contamination and allow suitable action to be taken as soon as the first signs of contamination become evident to avoid contamination of other cultures. Toxicity is not the only concern since hormones and other growth factors found in serum can cause changes those, while not necessarily harmful to cultures, may be unwanted by researchers using the system (Ryan ,1998; Freshney, 1981)). We isolated and cultured AF cells in different gestational age of buffalo. In this study we studied effect of various microbial contaminants i.e. bacteria, fungi and mycoplasma which inhibit the growth of cells regularly. In our study we found that during animal cell culture some bacteria such *S. epidermidis*, *S. aureus*, *B. subtilis*, *E. coli* (Fig.4B,5A)and fungi *S. cerevisiae* (fig. 4C, fig. A,B) affects the cell culture growth. Bacterial contamination was usually evident to the naked eye by a sudden increase in turbidity and color change (Fernando *et al*., 2005) of the culture medium (fig. 5A). As a result of change in pH (Cobo *et al*., 2005) though cells might survive for a short time, but they died eventually. Mycoplasmas are smaller than bacteria (0.3 µm in diameter) and can be observed as filamentous or coccal forms. There are several species which are known to occur in 98% of the laboratory infections of cell cultures: *Mycoplasma hyorhinis, M. arginini, M. orale, M. fermentans, M. salivarium, M. hominis and Acholeplasma laidawii* (McGarrity GJ, 1982). We also observed the mycoplasma contamination in our AF cell culture that lead to color change in the AF cell culture (fig. 5 B).

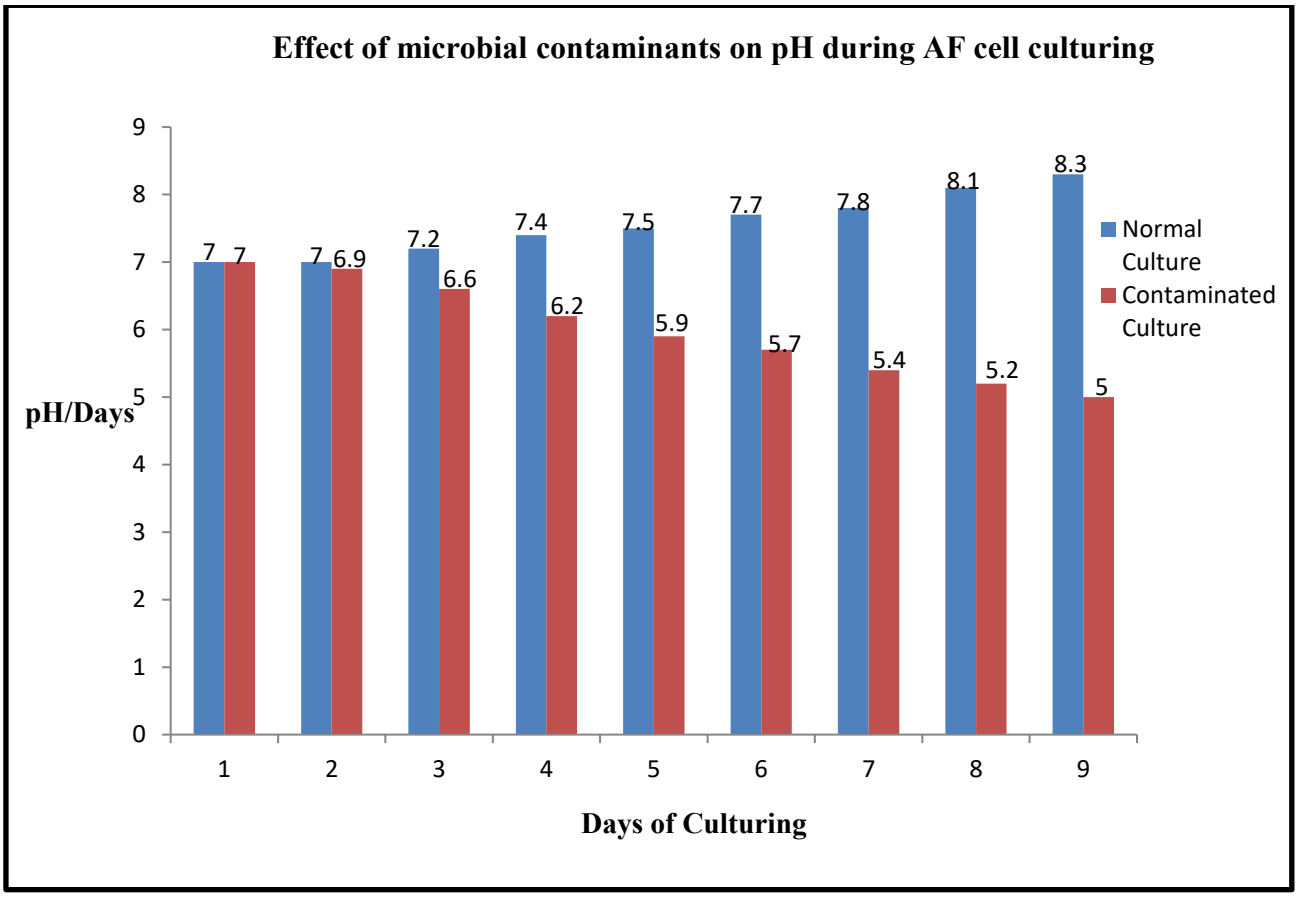

Fig.1 Effect of microbial contaminants on pH during A cell culturing

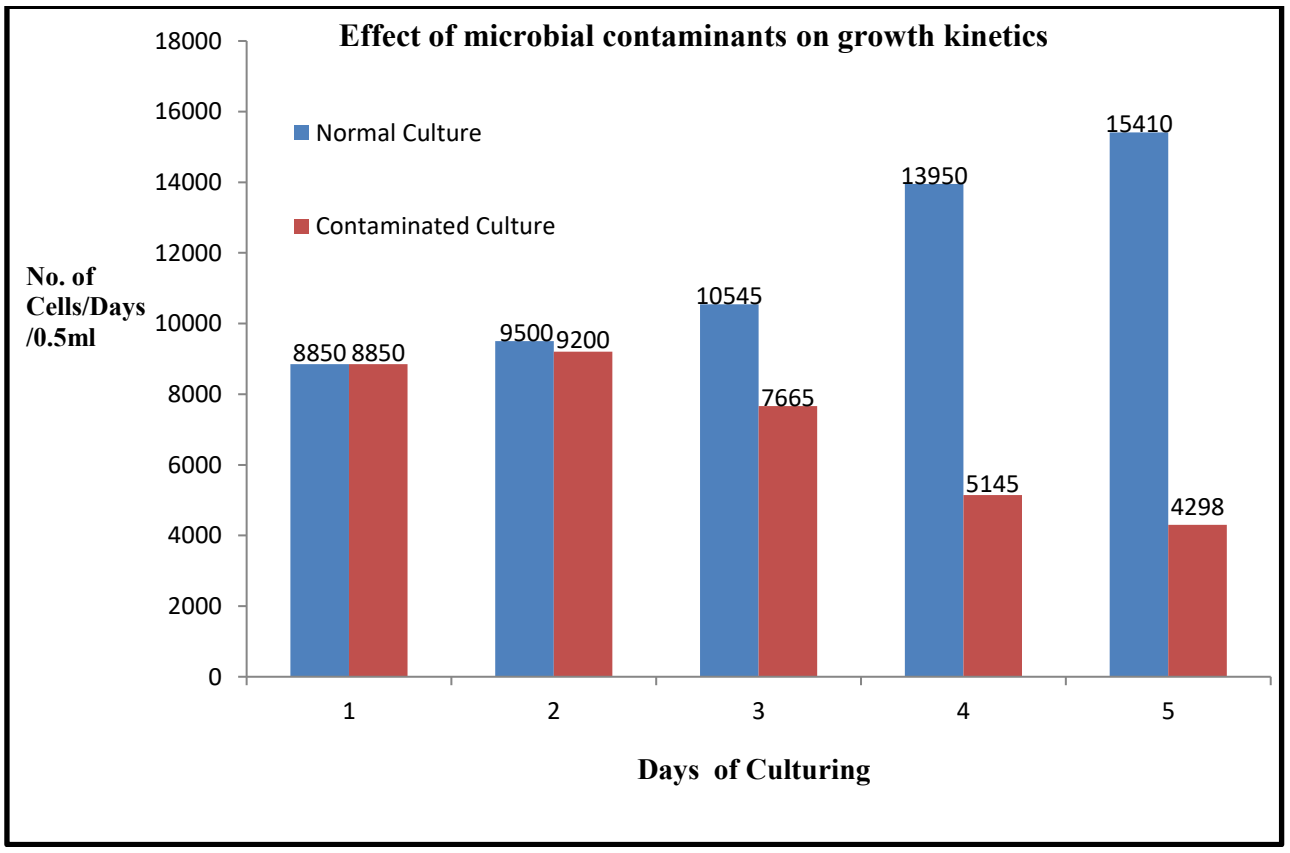

Fig. 2 Effect of microbial contaminants on growth kinetics of cell culture

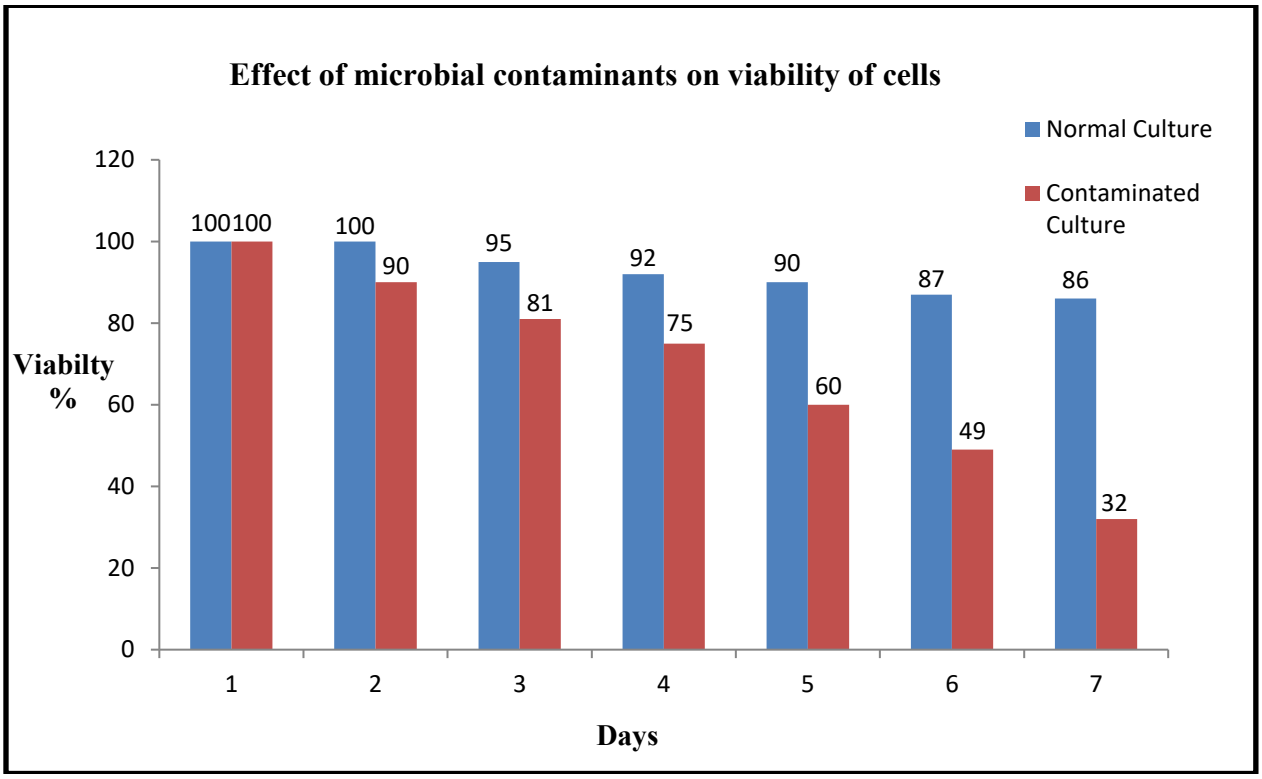

Fig. 3 Effect of microbial contaminants on viability of cells

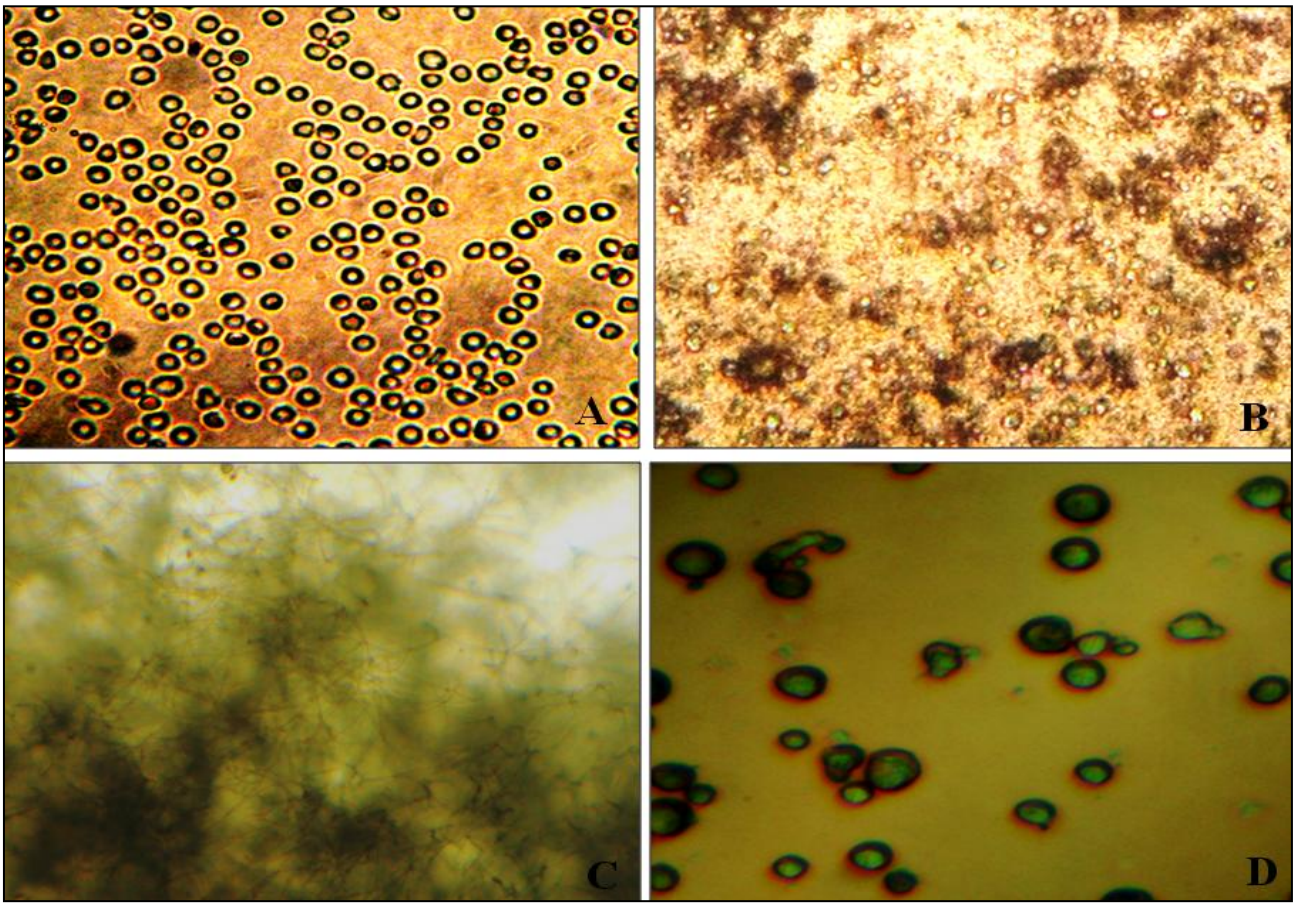

Fig. 4 Effects of microbial contaminants on AF cell culture. (A) Cell culture without any contamination, having normal cells, (B) culture medium having turbidity due to bacterial contamination, (C) culture medium having fungal growth and in (D) medium having mycoplasma growth (40X)

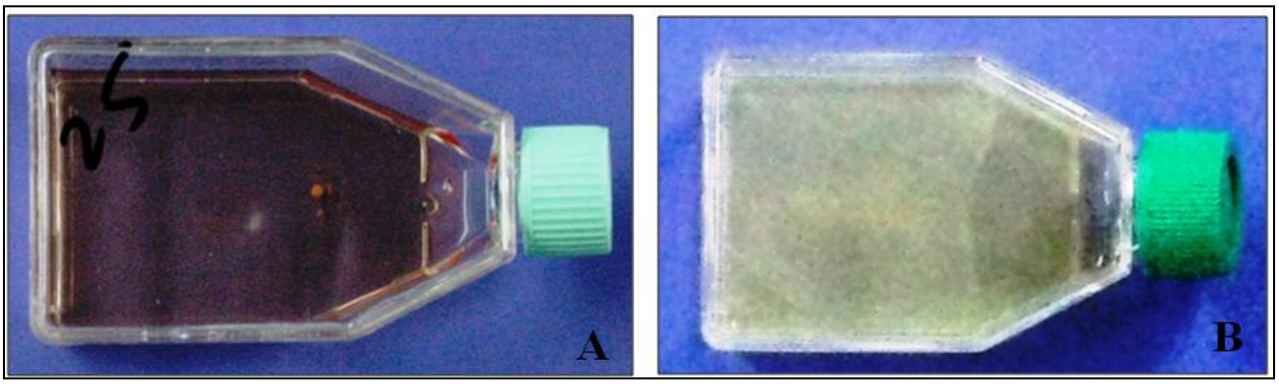

Fig 5 (A) Turbidity and change in the colour of the medium due to bacterial and (B) mycoplasma contamination

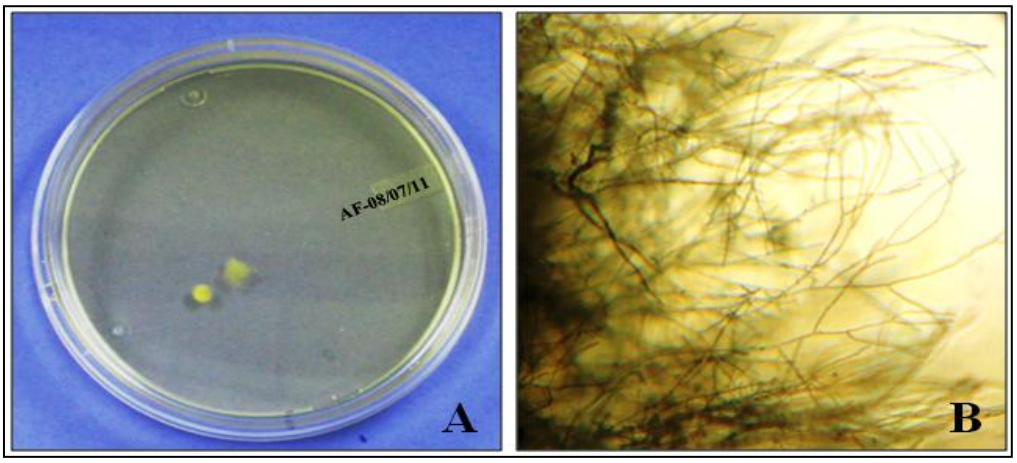

Fig 6 (A) Fungal growth in the 35mm petri plate and (B) thread like structure after microscopical investigation

From our study, we also found that microbial contamination affects the change in the pH of the medium from normal alkaline to acidic one (fig.1). Cells numbers becomes nearly half in the contaminated culture as compare to the fresh one (fig.2) and viability of the cells decreased from 100% to 32% within 5 days of culturing (fig.3). From overall results, we concluded that microbial contaminants fully affect the pH value of the culture medium, viability and cell numbers.

Acknowledgements

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