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Analysis & Trouble Shooting of the Contaminations, Arised in North Indian Zones during Plant Tissue Culture

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***Abstract*-** In the present investigation Jatropha and pea plant leafs was used, to germinated the plant by artificial condition through plant tissue culture method. We have taken three methods Method A, B & C to germinate callus, seed, bud and stem. 30 Sample were taken to analyze.
In Method A 60% samples were contaminated (fungus and bacteria) and 20% was shown growth.
In Method B 66% was contaminated by fungus and 19% were contaminated by bacteria and only 15% are shown growth.
In Method C 85% samples were contaminated by fungus and bacteria, only 15 % samples was show growth.
The study shows most of all contamination was due to Fungus, a very less contamination were due to bacteria. Among three method used with different Sterilization process. Method A was best suited for Callus Culture, Method B for Seed Culture & Method C for Stem & Bud Culture.

***Index Terms*-** Analysis of contamination occurs in plant tissue culture, Biotechnology, Graph for Stem Culture, callus culture, seed culture, bud culture.

I. INTRODUCTION

Biotechnology is name given to the methods and techniques that involve the use of living organisms like bacteria, yeast, plant cells etc or their parts or products as tools (for example, genes and enzymes). They are used in a number of fields: food processing, agriculture, pharmaceutics, and medicine, among others. Plant tissue culture can be defined as culture of plant seeds, organs, explants, tissues, cells, or protoplasts on nutrient media under sterile conditions.

Plant tissue culture, also referred to as cell, in vitro, axenic, or sterile culture, is an important tool in both basic and applied studies, as well as in commercial application *(Adams, R.P. and Adams, J. E. (eds.) 1992, Conservation of Plant Genes, DNA Banking and In Vitro Technology. Academic Press, New York.)*

Plant tissue culture is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions in vitro. The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902

on his experiments on the culture of single cells *(Bhojwani, S.S. (eds.) 1990, Plant Tissue Culture: Applications and Limitations. Elsevier, Amsterdam)*

He opined that, to my knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants have been made. Yet the results of such culture experiments should give some interesting insight to the properties and potentialities that the cell, as an elementary organism, possesses. Moreover, it would provide information about the interrelationships and complementary influences to which cells within a multicellular whole organism are exposed (from the English translation, *(Lewin, B. 2004 Genes VIII, Oxford University Press, New York)*

He experimented with isolated photosynthetic leaf cells and other functionally differented cells and was unsuccessful, but nevertheless he predicted that one could successfully cultivate artificial embryos from vegetative cells. He, thus, clearly established the concept of totipotency, and further indicated that the technique of cultivating isolated plant cells in nutrient solution permits the investigation of important problems from a new experimental approach. On the basis of that 1902 address and his pioneering experimentation before and later, Haberlandt is justifiably recognized as the father of plant tissue culture. Other studies led to the culture of isolated root tips *(Old, R.W. and Primrose, S.B. 1990.) (Singer, M. and Berg, P. 1991. Genes and Genomes, University Science Books, Mill Valley, California and Blackwell Science Publishers)*

II. MATERIALS AND METHODS

Reagents used:
Extran (5% v/v) / tween 20.
0.1 % (w/v) Mercuric chloride solution
0.7% (w/v) Agar

Growth regulators:N^6-benzyladenine (BA)/ kinetin (Kn) /indole-3-acetic acid (IAA)/indole 3- butyric acid (IBA) / α naphthalene acetic acid (NAA).
70 % Ethanol
1% Sodium hypochlorite

METHOD A - Young shoots and callus of jatropha plant as a source tissue were washed, first under running tap water followed by a detergent Extran (5%v/v) / tween 20 for 5 min. After thorough wash in water, source tissue was surface sterilized using 0.1 % (w/v) mercuric chloride solution for 7-9 min. After repeated washing with sterile water (3 times or 15 min) node

segment were cut into appropriate size (1.0-1.5 cm) and cultured on sterile nutrient medium

METHOD B -Capsules were washed with 2–3 drops of Tween 20 and surface-disinfected in 0.1 % mercuric chloride solution for 2 min followed by 70 % ethanol for 30 s and surface flaming. Finally, the capsules were rinsed five times with sterile distilled water and dried. All subsequent work was carried out aseptically in laminar flow cabinets. The capsules were then dissected longitudinally with a surgical blade and extracted seeds were spread thinly over the surface of liquid culture medium contained in 25 × 150 mm glass test tubes (20 test tubes per capsule) each containing 10 ml of medium

METHOD C- Take explants part of plant and wash with 2 drops of tween 20 around 15 minute and then washed the explants with sterile distilled water around 2 times and then dip the explants part in NaClO 10 % concentration and dip around 15 min and then washed up to 2 times and then planted on MS media

III. RESULTS AND DISCUSSION

Graph for Callus Culture

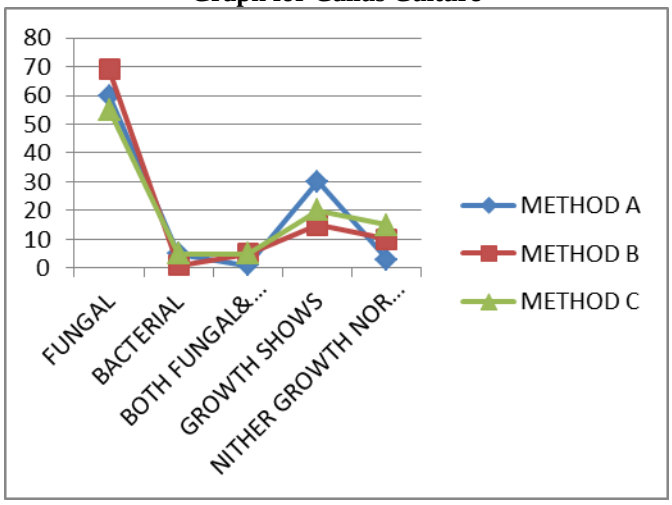

Fig 1: Graphical representation of contamination and growth in callus culture by all the three methods (method A, B and C)

Graph for Seed Culture

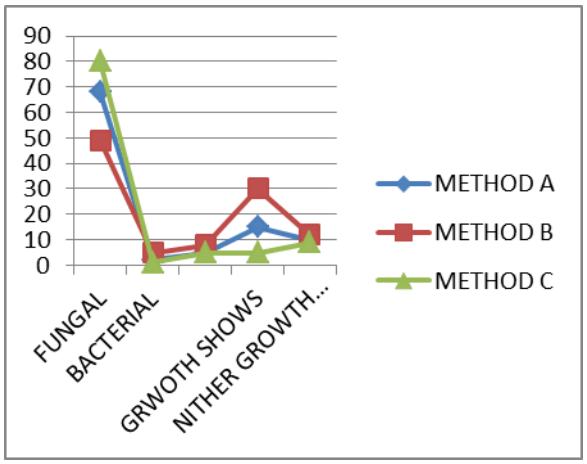

Fig 2: Graphical representation of contamination and growth in seed culture by all the three methods (method A, B and C)

Graph for Bud culture

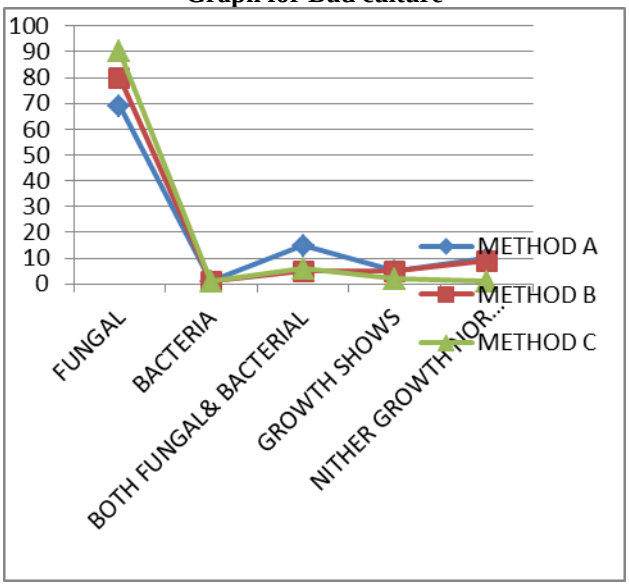

Fig 3: Graphical representation of contamination and growth in Bud culture by all the three methods (method A, B and C)

Graph for Stem Culture

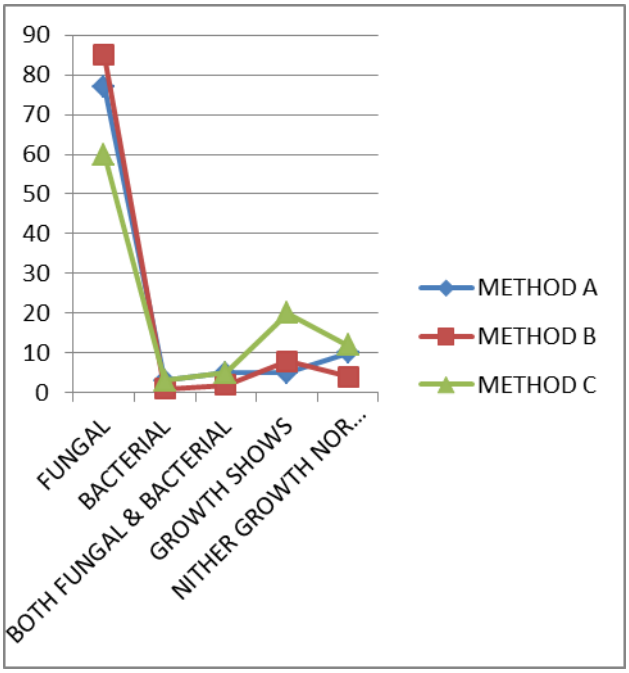

Fig 4: Graphical representation of contamination and growth in Stem culture by all the three methods (method A, B and C)

GRAPHICAL REPRESANTATION OF ALL THE CULTURES AND THEIR RESPECTIVE CONTAMINATION.

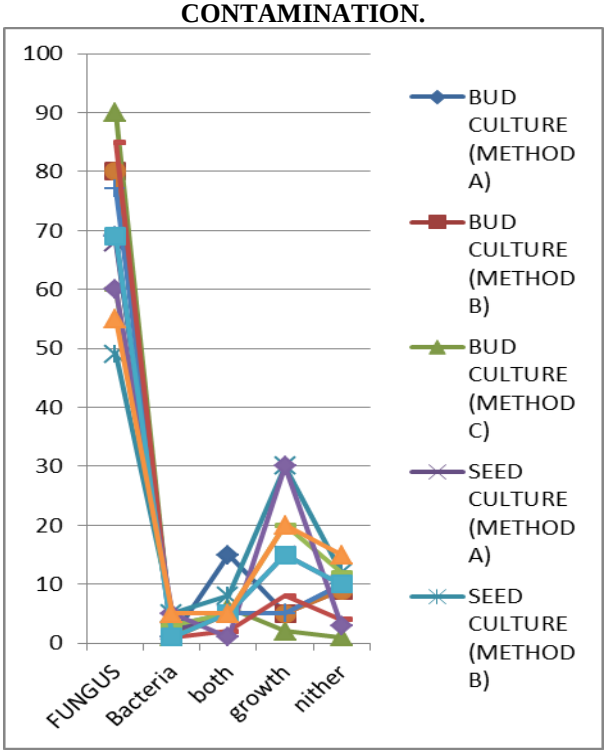

Fig 6: Graphical representation of contamination and growth in Callus culture, Stem culture, Bud culture and Seed culture by all the three methods (method A, B and C)

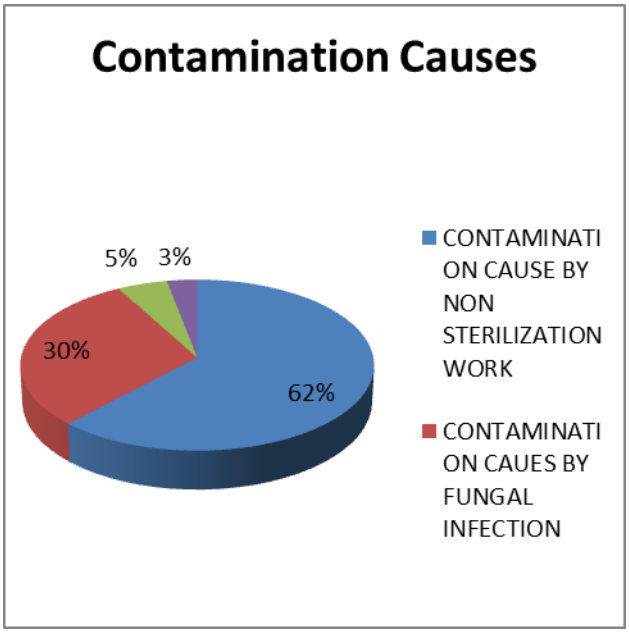

Fig 5: Pie chart depicting the percentage of contamination arising because of different reasons.

Above pie chart shows few other causes of contamination which we faced during our study. From this it is clear that in plant tissue culture field mostly contamination occurs when sterilization conditions are not maintained properly. Around 30 samples of callus cultures were taken, out of which 22 samples were clearly contaminated due to non sterilization conditions. And 30% samples were mostly contaminated by fungal infection, and 5% samples are mostly contaminated by lack of nutrition in MS media due to which other growth inhibits the growth of samples. And 3 % samples were mostly contaminated or died due to overheating of the samples in white light.

IV. CONCLUSION

The ability to establish and grow plant cell, organ, and tissue cultures has been widely exploited for basic and applied research, and for the commercial production of plants (micro-propagation). Regardless of whether the application is for research or commerce, it is essential that the cultures be established in vitro free of biological contamination and be maintained as aseptic cultures during growth and storage successful micro propagation.

Contamination in plant tissue culture could have very serious consequences on the result. These contaminations could originate from any of the sources as discussed above. Besides fungal and bacterial contamination, non sterilization work plays a major role in causing contamination in plant tissue culture work. In this study we focused mainly upon the percentage of fungal contamination, bacterial contamination, contamination caused by non sterilization work and contamination caused by loss of nutrition.

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