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Microbes as a Hurdle of Plant Tissue Culture and Precautions – A Review Article

Joshi K. R., Suthar R. S. and Kahrizi Danial*
Assistant Professor, Department of Biotechnology, P. S. Science & H. D. Patel Arts College, kadi-382517. H.N.G.University, Patan, Gujarat, India.
***Professor, Department of Plant Production and Genetics, Razi University, Kermanshah, Iran.**

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Corresponding Author Email: ram_v2@yahoo.com

Abstract
Microbes are hurdle of plant tissue culture; it is greatest problem of plant tissue culture techniques. Contaminates such as fungal and bacteria were reduce plant growth and die explants in media. All types of steps and techniques were described in Research review. Microbial contamination is a continuous problem, which frequently compromise development of all in vitro techniques. This study aimed to focus all types of contaminates and steps to reducing contamination in laboratory. Sterilization is main step to avoid contaminates in media. Antibiotics and antifungal agents are capable to avoid contaminates on the surfaces of explants and in media.

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INTRODUCTION
Plant tissue cultures mainly face lot of contamination of microbes such as bacteria, fungus, many of species due to handling and error of sterilization process. It is very big hurdle of plant tissue culture. Investigators try to avoid all problems. Contamination reduces plant growth and in Commercialization of tissue cultures its big problems. Bacterial contamination can reduce growth rate, retard rooting, and even cause plant death (Leifert and Waites, 1992). Microorganisms (filamentous fungi, yeast, bacteria, viruses and viroid's) and microarthropods (mites and thrips) have been identified as contaminants in plant tissue cultures. Tissue cultures can become contaminated at any stage of the tissue culturing process (Leifert, 2000). During micro propagation, bacterial contamination can remain undetected because the salt concentration, sucrose concentration, pH and temperature are not optimal for bacterial growth (Cooke et al., 1992). Fugues and bacteria are difficult to control because growth of microbes is very fast than plant. Contamination is cost effective factor to avoid by practices in plant tissue culture labs and industries. It is a hurdle to prevent and growth healthier plants. Some effective practices were done in laboratory to prevent. One more problem of endogenous microbes, which were not, appears any symptoms and hidden contamination in cultures. Plant tissue cultures can become contaminated with bacteria either because of poor aseptic technique while handling the cultures or because of animal vectors (Leifert *et al*., 1994).

PLANT AND EXPLANT MATERIALS
Selection and collection of plant materials should be important with different criteria. Mother plant should be young and healthy. Genetically healthier plants were best selection of fast growth. Resistance and tolerant plant was selected in criteria. Surface sterilization is most prominent practices to prevent microbe's contamination in culture. Different surface sterilizers were used to avoid contamination described in table-1. Surface sterilization of different explants of plant material were established by

various investigators and used different surface disinfectors (Kahrizi and Soorni 2013; Ebrahimie *et al*., 2007; Tawfik and Noga, 2002; Suthar, R. *et al*., 2014).

Table: 1. Different surface sterilizers use to prevent contamination in plant cultures

Sr. No.	Surface sterilizer	Concentration	Treatment (Min)
1.	$HgCl_2$	0.01-1%	1-3 min
2.	Fungicide	2-5 gm / lit	15-20 min
3.	Tween 20	1-2 drop / 100 ml	5-10 min
4.	NaOCl	1-1.4%	5-20 min
5.	Antibiotics	4-50 mg / lit	20-30 min

MEDIA AND CHEMICALS

Plant tissue culture media autoclave properly with standards protocol 121^{0}C temp, 15-psi pressure for 15-20 min. all chemicals and growth regulators should be either filter sterilized or autoclaved. To put off contamination in media or basal contamination media must be autoclaved and culture vassals too. Some antibiotics and antifungal were use in media to avoid contamination in it as describe in table-2 and 3. Antibiotic stock solutions were made freshly every day, filter sterilized, and added to the medium after autoclaving (Wojtania A. & Gabryszewska E. 2001). Antibiotics and antifungal were used in either plant tissue culture media or surface sterilization process.

Table: 2. Antibacterial agent's use in plant tissue cultures

Sr. No.	Antibacterial Agents	Effectiveness
1	Amoxycillin	Bactericidal for both positive and gram negative
2.	Hygromycin B	Highly active against bacteria, fungi and higher eukaryotic cells
3.	Cefotaxime	gram negative

Table: 3. Antifungal agent's use in plant tissue cultures

Sr. No.	Antifungal Agents	Concentration
1.	Amphotericin	5mg / lit
2.	Nystatin	40mg/lit
3.	Topsin M 70 WG	70-100 mg / lit
4.	Bravo 500 SC	0.5-0.75 ml / lit
5.	Sportak 45 EC	0.5-0.8 ml / lit

LABORATORY PREPARATION

Aseptic condition was maintained in plant tissue culture laboratories. Fumigation and spray were good practices to avoid contamination in lab once a week and high load of work it should be spray alternate day. LAF must be clean and spray with 70% ethyl alcohol. UV light is alternative option to avoid contamination and provide aseptic condition in LAF as describe in table-4.

Table: 4. Fumigation and spray use in plant tissue cultures

Sr. No	Fumigation / Spray	Treatment
1.	Spray- LAF	70% ethyl alcohol
2.	Spray -LAF	10% Zonrox
3.	Fumigation-LAB	$KMNO_4$ and formaldehyde
4.	Fumigation- LAB	H_2O_2 and chlorine dioxide
5.	UV- LAF	Exposure 30-40 min before use

Three different fumigant agents were used in the Amerithrax 2001 remediation paraformaldehyde, gaseous chlorine dioxide and hydrogen peroxide vapour (H_2O_2) (Canter *et al*. 2005).

HANDLING AND INOCULATION

Handling and inoculation skills are very important to stay away from contamination in cultures. Not allow any extra materials in LAF. All materials must be sterilized. Gloves mask and cap must be wear. Inoculated explant under LAF Precisely. Handling avoids contaminations described in table-5.

Table: 5. Precaution in plant tissue cultures

Contamination	1. Validate autoclave 2. Sterilization time should be strictly as per standard 3. Close the sterile container properly to prevent entry of non-sterile air 4. Medium should be preferably handled by trained 5. Proper aseptic techniques should be observed

CONTAMINATION IN CULTURES

Microbe growth is fast than plants cells and tissues. In Twelve to 24 hrs bacteria and fungus were contaminated the cultures. Fungus was appeared first because of appropriate temperature in culture room. Contamination visible on media, in media and on explants surface (table-6).

Table: 5. Fungal contamination in plant tissue cultures

Contaminants	Cultural characteristics	References
Aspergillus sp.	Colonies are flat, circle, filamentous, velvety, wolly or cottony texture. Colony color is gray to green	Jojine S. C. and Alminda M. F., 2016. Kowalik., 2008
Fusarium equseti.	Colonies are off white hyphae to dark pink reverse due to pigment production	Suthar, R. S. & Bhatt, P. N. 2012.
Fusarium oxysporum	Colonies are off white	Lawson and Hsu, 1996
Alterneria tenuis & *Alterneria. longipe*	Colonies are fast growing, black to olivaceous-black or greyish	MORTON, F. J. 1964
Cladosporium	Colonies are slow growing, mostly olivaceous-brown to blackish-brown but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia.	Kowalik., 2008
Penicillium	Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores.	Kowalik., 2008
Gliocladium	*Gliocladium* is often described as a counterpart of *Penicillium* with slimy conidia. Colonies are fast growing, suede-like to downy in texture, white at first; sometimes pink to salmon, becoming pale to dark green with sporulation.	Farr et al. 1996

The bacterial contaminants include *Pseudomonas flourescens, Escherichia coli, Proteus* sp*, Micrococcus* spp*, Streptococcus pneumoniae, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Corynebacterium* sp *and Erwinia* sp.

CONCLUSION

Microbes such as bacteria and fungus are most hurdles for plant tissue culture, but lot of technologies are available to reducing contamination. Plant tissue culture research was affected to contamination. Research review focuses all possible ways and techniques to avoid contamination and reduce time and cost. Review would be economically important. Several logical protocols could be taken greatly reduce fungal and bacteria contaminates in plant tissue culture. Plant tissue culture technology is being commonly used for mass production and plant multiplication, the greatest problem in plant techniques is contaminations. All possible steps and chemicals were described.

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