

***In vitro*, effect of sucrose concentration and pH on MS medium contaminants**

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Abstract

A study was conducted in the laboratories of College of Agriculture and Marshlands, Department of Horticulture and Landscape Engineering, University of Thi Qar, Iraq. During the period from February to May 2024. Aims of a study to isolate and diagnose the most important fungi that infect agricultural media in tissue culture laboratories, and tested to effect of sucrose and pH in MS medium on the growth and development of contaminated fungi. The results confirmed that there is an effect of sucrose concentration added to the tissue culture medium and the acidity of the medium on the growth and development of fungal colonies, as 0 g.L⁻¹ concentration did not record any fungal growth, while high concentrations of sucrose showed significant growth for all species under study. Also, a study showed that there is a significant effect of pH medium on the growth and development of fungal colonies depended the degree of response varies according to the type of fungus, and there were an interaction between the effect of pH medium with sucrose concentration, as the response of fungal species to this interaction differed towards inhibiting the growth of colonies despite the increase in the concentration of sucrose.

Keywords: *Tissue culture, explants, colonies, medium, fungi.*

I. Introduction

Plant tissue culture is a special method of propagation when traditional propagation methods are unavailable or non-existent, especially in the field of producing cloned strains and maintaining the desired genotype. This technique is characterized by complete control over the laboratory environment and the amount of nutrients required for plant growth (Bhatia, 2015). Plant propagation by tissue culture is done using different types of Explants such as organs culture (shoot tip culture, nod tip culture, levies culture and roots culture), anthers culture, cell suspension and embryos culture (Alghasheem, et al., 2022). Plant tissue culture techniques face many challenges, including contamination with fungal and bacterial pathogens. Sugars in media are essential nutrients for the development of fungal pathogens in tissue culture because these receptors are used as carbon structures for the biosynthesis of other compounds as well as as substrates for energy production and are involved in cellular signaling pathways in fungi (Goulet and Saville, 2017). It has been shown that high levels of sugars may affect extracellular wall-degrading enzymes secreted by fungal pathogens. These enzymes may contribute to pathogenesis by degrading wax, cuticle and cell walls, thereby aiding tissue invasion and pathogen spread (Kikot et al., 2009). A pH in plant tissue culture is an important factor affecting nutrient uptake, plant growth and development and the growth of fungal contaminants. Many plant species grown in tissue culture media can tolerate a wide range of pH values in the range 4.0-7.2. Many studies have shown that the best growth results are usually obtained using a low-nutrient, acidity medium 5.8-5.8 (George et al., 2008). Aims of a study to tested to effect of sucrose and pH in MS medium on the growth and development of contaminated fungi.

II. Materials and Methods

Culture media, Isolation and identification of fungal

A study was conducted in the laboratories of College of Agriculture and Marshlands, Department of Horticulture and Landscape Engineering, University of Thi Qar, Iraq. During the period from February to May 2024. Aims of a study to tested to effect of sucrose and pH in MS medium (Murashige and Skoog, 1962) on the growth and development of fungi contaminated. A study was carried out via placing MS culture media (pH = 5.5) in petri dishes, with added Amoxicillin antibiotic (500 mg/L) to the culture medium to inhibit bacterial growth, then cultures media were exposed to air in the laboratory two days to allow fungi spores to grow then we were placed in the incubator at a temperature of (25+₋ 2) °C for 10 days. After 10 days of incubation, the fungal colonies growing in the plant tissue culture medium MS were studied and identified by Serial Dilution method was used for spores obtain pure fungal growths (Amoghavarsha et al., 2022).

PDA culture medium was prepared by autoclaving at a temperature of 121 °C and a pressure of 15 pounds for 20 minutes (Benyan, 2024). The fungal species were transferred to PDA culture medium and incubated at a temperature of (25 + 2) °C for 5-7 days and then stored in the refrigerator until use. Based on referces taxonomic, the isolated fungal colonies were classified and identified through glass slides containing Lactophenol cotton blue (LCB) under the light microscope (Booth, 1966, Agrios, 2004).

For tested effect of sucrose and pH on the growth and development of fungal colonies in tissue culture media, fungal colonies with a diameter of 2 mm were taken from four types of fungi under the condition of *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.* And *Fusarium spp.* study and cultured in Petri dishes containing liquid MS medium containing 4 different concentrations from sucrose as a carbon source (0, 5, 10 and 20) g.L⁻¹ and four levels of pH 4, 5, 6 and 7) and then placed in the incubator at a temperature of (25+₋ 2) °C for 10 days the results were recording after 10 day by readings dry weight of the fungus. The experiment was conducted using a completely randomized design (C.R.D) and was repeated three times. The results of the experiment were statistically analyzed by estimating its dry weight using the GENSTAT statistical analysis program, where the two-way analysis of variance test and the least significant difference (LSD) test were used at a probability level of 0.01%.

III. Results and discussion

1.Effect of sucrose concentration and pH on dry weight of *Aspergillus spp.* contaminants

The results showed that there were 4 main types of Qatari colonies that were diagnosed growing in MS medium (*Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.* and *Fusarium spp.*). The results of the statistical analysis of the experiment in Table (1) showed significant differences between sucrose and pH on the dry weight (g) of *Aspergillus spp.* fungi growing in tissue culture medium. 5 g.L⁻¹ sucrose concentration recorded the lowest dry weight value for the fungus, which amounted (0.1857) g compared to 20 g.L⁻¹ sucrose concentration, which recorded the highest dry weight value for the fungus, which amounted (1.6663) g, while 0 g.L⁻¹ sucrose concentration did not record any growth (0.0000) g. Also, results was showed pH=7 recorded the lowest dry weight rate (0.4891) g, while pH=5 recorded (0.9134) g. Also, results there was interaction between sucrose and pH were recorded significant differences, where the concentration of 5 g.L⁻¹

sucrose concentration and PH=7 recorded the lowest dry weight rate (0.0357) g compared to a 20 g.L⁻¹ sucrose concentration and pH=5, where the highest dry weight value of the fungus was recorded at (2.1103) g.

Table (1) Effect of sucrose and pH on dry weight (g) of *Aspergillus spp.* growing in tissue culture medium after 10 days from incubation

pH	Sucrose g.L ⁻¹				Mean of pH
	0	5	10	20	
7	0.0000	0.0357	0.7120	1.2087	0.4891
6	0.0000	0.1207	0.8473	1.8393	0.7018
5	0.0000	0.3523	1.1910	2.1103	0.9134
4	0.0000	0.2340	0.9030	1.5067	0.6609
Mean of Sucrose	0.0000	0.1857	0.9133	1.6663	
LSD	For Sucrose 0.06705	For pH 0.06705		For interaction 0.13409	

2. Effect of sucrose concentration and pH on dry weight of *Penicillium spp.* Contaminants

The results of the statistical analysis of the experiment in Table (2) showed significant differences between sucrose and pH on the dry weight (g) of *Penicillium spp.* fungi growing in tissue culture medium. 5 g.L⁻¹ sucrose concentration recorded the lowest dry weight value for the fungus, which amounted (0.0843) g compared to 20 g.L⁻¹ sucrose concentration, which recorded the highest dry weight value for the fungus, which amounted (1.1427) g, while 0 g.L⁻¹ sucrose concentration did not record any growth (0.0000) g. Also, results was showed pH=7 recorded the lowest dry weight rate (0.3873) g, while pH=5 recorded (0.5986) g. Also, results there was interaction between sucrose and pH were recorded significant differences, where the concentration of 5 g.L⁻¹ sucrose concentration and PH=7 recorded the lowest dry weight rate (0.0250) g compared to a 20 g.L⁻¹ sucrose concentration and pH=5, where the highest dry weight value of the fungus was recorded at (1.3167) g.

Table (2) Effect of sucrose and pH on dry weight (g) of *Penicillium spp.* growing in tissue culture medium after 10 days from incubation

pH	Sucrose g.L ⁻¹				Mean of pH
	0	5	10	20	
7	0.0000	0.0250	0.5093	1.0150	0.3873
6	0.0000	0.0980	0.6317	1.1280	0.4644
5	0.0000	0.1320	0.9457	1.3167	0.5986
4	0.0000	0.0823	0.4120	1.1113	0.4014
Mean of sucrose	0.0000	0.0843	0.6247	1.1427	
LSD	For Sucrose 0.02751	For pH 0.02751		For interaction 0.05502	

3. Effect of sucrose concentration and pH on dry weight of *Rhizopus spp.* Contaminants



Results of experiment the statistical analysis in Table (3) showed significant differences between sucrose and pH on the dry weight (g) of *Rhizopus spp.* fungi growing in tissue culture medium. 5 g.L⁻¹ sucrose concentration recorded the lowest dry weight value for the fungus, which amounted (0.0333) g compared to 20 g.L⁻¹ sucrose concentration, which recorded the highest dry weight value for the fungus, which amounted (0.3093) g, while 0 g.L⁻¹ sucrose concentration did not record any growth (0.0000) g. Also, results was showed pH=4 recorded the lowest dry weight rate (0.0165) g, while pH=6 recorded (0.2588) g. Also, results there was interaction between sucrose and pH were recorded significant differences, where the concentration of 5 g.L⁻¹ sucrose concentration and pH=4 recorded the lowest dry weight rate (0.0106) g compared to a 20 g.L⁻¹ sucrose concentration and pH=6, where the highest dry weight value of the fungus was recorded (0.6123) g.

Table (3) Effect of sucrose and pH on dry weight (g) of *Rhizopus spp.* fungi growing in tissue culture medium after 10 days from incubation.

pH	Sucrose g.L ⁻¹				Mean of pH
	0	5	10	20	
7	0.0000	0.0303	0.2296	0.6123	0.2180
6	0.0000	0.0680	0.4453	0.5220	0.2588
5	0.0000	0.0243	0.0360	0.0623	0.0306
4	0.0000	0.0106	0.0150	0.0406	0.0165
Mean of sucrose	0.0000	0.0333	0.1815	0.3093	
LSD	For Sucrose 0.00514	For pH 0.00514		For interaction 0.01028	

4. Effect of sucrose concentration and pH on dry weight of *Fusarium spp.* Contaminants

Results of experiment the statistical analysis in Table (4) showed significant differences between sucrose and pH on the dry weight (g) of *Fusarium spp.* fungi growing in tissue culture medium. 5 g.L⁻¹ sucrose concentration recorded the lowest dry weight value for the fungus, which amounted (0.0547) g compared to 20 g.L⁻¹ sucrose concentration, which recorded the highest dry weight value for the fungus, which amounted (0.2437) g, while 0 g.L⁻¹ sucrose concentration did not record any growth (0.0000) g. Also, results was showed pH=4 recorded the lowest dry weight rate (0.0572) g, while pH=5 recorded (0.1627) g. Also, results there was interaction between sucrose and pH were recorded significant differences, where the concentration of 5 g.L⁻¹ sucrose concentration and pH=4 recorded the lowest dry weight rate (0.0130) g compared to a 20 g.L⁻¹ sucrose concentration and pH=5, where the highest dry weight value of the fungus was recorded (0.3153) g.

Table (4) Effect of sucrose and pH on the dry weight (g) of *Fusarium spp.* fungi growing in tissue culture medium after 10 days from incubation

pH	Sucrose g.L ⁻¹				Mean of pH
	0	5	10	20	
7	0.0000	0.0136	0.0843	0.2413	0.0848
6	0.0000	0.0753	0.1336	0.2750	0.1210
5	0.0000	0.1170	0.2186	0.3153	0.1627
4	0.0000	0.0130	0.0726	0.1433	0.0572
Mean of sucrose	0.0000	0.0547	0.1273	0.2437	

LSD	For Sucrose 0.00573	For pH 0.00573		For interaction 0.01147	
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Results in Tables (1, 2, 3 and 4) showed that there were significant differences between fungi contaminated and sucrose concentrations added to MS medium, 0 g.L⁻¹ sucrose concentration did not record any growth like *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.* and *Fusarium spp.* despite the availability of all major and minor mineral elements and vitamins in culture medium, while high concentrations of sucrose recorded the highest growth of fungal colonies for all species. The reason for this is that sugars are essential nutrients for the development of fungal pathogens in tissue culture technology because these metabolites are used as carbon structures for the biosynthesis of other compounds in addition to being production substrates. Energy and participate in cellular signaling pathways in fungi. The results of this study was similar to studies of (Formela - Lubońska et al., 2020) to determine the relationship between the levels of sucrose, glucose or fructose in the growth of the fungus *Fusarium oxysporum*. The results of the growth analysis of *Foxysporum* fungi in water agar showed that the addition of soluble sugars 60 mmol of sucrose and 120 mmol of glucose significantly stimulated the growth of this fungus compared to the control samples. Also, results study of (Hamad et al., 2014) to determine the growth of *Aspergillus niger* on two culture media PDA to maintain a pure strain of the fungus and Czapeck Dox Agar to test the effectiveness of sugars and the effect of carbon element on the growth of the fungus using five different carbon sources (glucose, fructose, sucrose, maltose and starch) are also similar, while the control sample lacked any added sugars, the study found that *Aspergillus niger* differs in its ability to utilize added carbon sources. Fructose and sucrose were found to be suitable sources of carbon for fungal isolates, while starch, as a polysaccharide, was a poor source of carbon for *Aspergillus niger* growth.

Also, the results in Tables (1,2, 3 and 4) showed that there were significant differences between pH and fungi contaminated with in MS medium, as the response of fungi contaminated to the species under study differed at pH (7,6,5 and 4). This is attributed to the fact that fungi are able to grow and develop over wide ranges of acidity. Many species can actively adjust the acidity of their environment by secreting acids or alkalis (Landraud et al., 2013). The ability to control extracellular pH is an important aspect of fungal physiology that contributes to host compatibility (Vylkova et al., 2017). Studies indicate that the pH for fungal growth ranges from 3 to 8 with maximum dry weight production of fungi in the liquid medium (Deshmmukh, 2012).

CONCLUSION

Results confirmed that there is an effect of sucrose concentration added to the tissue culture medium and the acidity of the medium on the growth and development of fungal colonies, as 0 g.L⁻¹ concentration did not record any fungal growth, while high concentrations of sucrose showed significant growth for all species under study. Also, a study showed that there is a significant effect of pH medium on the growth and development of fungal colonies depended the degree of response varies according to the type of fungus.

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