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EFFECT OF HONEY IN THE STERILIZATION OF EXPLANT *IN VITRO*

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Abstract

In this experiment, honey was tested in the medium used in plant tissue culture as an antibiotic and for the production of a medium resistant to contamination and tested its effectiveness without passing traditional sterilization stages without passing autoclave phase using concentrations (1,2,3,4,6,8) g/L without autoclave and then was incubation with different explants (seeds, shot tip, callus) of castor plan. The results showed that the addition of honey to medium has a significant effect in the conservation medium of contamination without use of autoclave at the concentrations (4, 6, 8) g / L of honey where there was no contamination during the incubation period 20 days and the same concentrations that have not contaminated after incubation of the explants. While the autoclaved medium did not contaminate at all the honey concentrations, the high concentrations effected in direction explants growth and tissues in the concentrations (6, 8) g / L failed in seed germination, rooting shoot tip growth and stimulated callus growth while concentration did not appear 4g/L impact on the direction of growth in addition to being a medium resistant to contamination.

***Keywords*:** Cytogenetic, Tissue culture and Molecular biology lab.

Introduction

The problem of contamination is one of the main problems facing tissue culture, and because of this, many measures are taken for sterilization of the medium and the explants, and many sterilization procedures and preventive measures to transfer sterile explants to the sterile medium in an environment devoid of pathogenesis (Hussein and Khierallah, 2013). These complex procedures increase the costs of production in tissue culture as well as is considered an influential factor in scientific experiments and the sterilized materials have an effect on the damage of the plant part and on the progress of production and scientific experiments Despite all these costly, procedures do not leave many factors and laboratories without percentage of contamination and some parts of the plant is the cause of internal contamination cannot be eliminated by surface sterilization and may appear after the incubation of the explants on medium after several days of inoculation.

The addition of antibiotics has had an effective effect on the disposal of the internal contamination of the explants, but not all contaminants in this particular case include the internal contamination of the explants or contaminants after inoculation (Tambarussi, 2015).

Although some types of honey have a high ability to kill germs, they generally kill germs and have the ability to prevent the growth of germs, which is an antimicrobial in the wounds (Dixon, 2003) (Molan, 2001) (Kings, 2001) The use of honey since antiquity, as studies and studies have confirmed the susceptibility of honey to the elimination of laboratory microbes in the treatment of wounds due to physical and chemical properties such as acidity, acidity and anti-inflammatory (Molan, 2002), and that 10% of honey has an effective effect in (2002). In a study of bacteria in the mouth due to the properties of antibacterial honey, it proved effective in eliminating germs (Molan, 2001). It also proved the effectiveness of honey in the treatment of stomach infections in particular and the digestive system in general (Gharzouli *et al*., 2001; Bilsel *et al*., 2002; Aysan *et al*., 2002; Al-waili, 2001). In addition to the fact that honey is vital in the treatment of decay, bacteria that cause bad breath and peptic ulcers, it can be used as an antibiotic in tissue culture and producing a pollution-free medium if used in quantity The experiment aimed at determining the best concentration added to the medium, making the medium free of contaminants and not affecting the process of experiments significantly and proved its ability to rooting (Mehta, 2013) has stimulated honey cutting rooting more than 90% (Federal, 2017) as a natural hormone for rooting and can stimulate the formation of callus in high concentrations and dispense with the use of autoclave because this step expensive because of the cost of the autoclave and what it needs to run, seriousness of the work of this device, To the destruction of many chemical compounds and growth regulators through high temperature and high pressure, which is broken or converted to other compounds not required (Ibrahim, 2017).

Materials and Methods

In this experiment, honey was tested for resistance to pollution or as an antibiotic in tissue farms using autoclave or without an autoclave. Plant cultivation was also tested on nonsterile medium**.**

Honey was obtained from bee farms belonging to Dr. Hadi Abdul Jalil, one of the experimenters to ensure that honey is free of any commercial fraud or additives. Honey was added to the medium various concentrations (1, 2, 3, 4, 6, 8) g/L. In addition to the comparison treatment, the percentage of contamination of the media was calculated without the use of autoclave after five days, after ten days and then fifteen days. After 20 days, the same calculations were carried out after the autoclave sterilization. The percentage of contamination was increased after inoculation with the explants. The percentage of contamination was calculated without sterilization of the explants and extent of the resistance of the medium, which was supplied with various honey concentrations, was contaminated at different intervals. To determine the effect of honey in the medium as a hormone, three explants (seeds, shoot tip, callus) were tested on the eight concentrations as well as the comparison treatment for 28 days.

The experiment was designed by Randomized Complete Design, in ten replicates for each treatment the mean was tested according to the selection of the lowest difference mean Least Revised Statistical Analysis Program and Use 5% Probability and Level Significance Difference (R.L.S.D) Genstat (2007).

Results and Discussion

This experiment was carried out in the laboratory of plant tissue culture in the Department of Botany, Al-musiab technical college Al-furat Al-awsat technical university for the purpose of determining the best concentration of honey acts as an antibiotic to resist contamination.

Data shown in table (1) that the medium that does not contain honey contaminated in the first five days, while the rest of the concentration in the percentage of contamination, The percentage of contamination at the end of the period was less than 2 g / L. The percentage of contamination at the concentration 3 is acceptable, but the best contamination ratio was achieved at the concentration of (4, 6, 8) g /l.

This shows the susceptibility of honey to resist contamination and the absence of honey full trinity decreased the proportion of contamination by increasing the amount of contamination.

Table 2 shows that the efficiency of sterilization in autoclaves in all concentrations is constant because the effect factor is autoclave sterilization, no effect of honey factor and no effect of the contamination factor to prove sterilization efficiency on all concentrations in the same conditions**.**

From the third table, it is found that the inoculation of non-sterilized explants in the medium of the sterile sterilized plant was not effective in adding the honey at the first, second and third concentration. However, it was included between the complete contamination in the comparison treatment 100 to 88.7, 78.2 and finally 33.5 at concentration 3, 6.7 This indicates the ability of honey to act as an antibiotic in delaying the contamination of explants and it has the ability to prevent contamination in high concentrations so that there was no contamination in the concentrations 6 and 8 g/l Although the explants were contaminated and forced to sterilize the medium, but did not find Usefulness in overcoming contamination.

In this table No. 4, honey works as a resistance to contamination. It also works as an antibiotic. The control treatment, which has a pollution ratio of 33.6, is resistant to contamination from the first concentration 28.7 and was acceptable in the second concentration 11.3 which did not differ significantly in the third concentration, contamination at the fourth concentration may cause special contamination in the medium and below the average efficiency. However, honey works to resist contamination and acts as an antibiotic to resist the internal contamination of the parts of the plant that is not served by surface sterilization. Selection of different explant (seeds, shoot tip, and salsa) to determine the effect of the honey medium on the growth direction and growth of the explants.

Note from Table 5 that the amount of callus was not affected in the treatment of control, while the effect of honey added to the medium, but not the significantly form on the soft weight of callus 107 g did not differ significantly until the fourth concentration 128 g and then jumped to 178 g at the concentration 6 g, and then to 288 g at the concentration of 8 g The increase in the weight of callus in the lower concentrations are considered in the natural limits being affected by an increase in the number of carbohydrates or an increase in energy, while the increase in the last two concentrations (6 and 8) g/liter natural border Fidel on The hormonal effect of honey (Mariola *et al*., 2019; Kapil *et al*., 2017).

In this table (6), we have adopted observations for each case and have been evaluated with general suspicion. In the control treatment, we note that the germination was normal and was not affected until the concentration of 6 g where the seeds started to bulge only, had an effect on germination but could not act as a catalyst for callus formation while honey inhibited the growth of the seed embryo and acted as a hormone to be callus of the embryo (Mariola *et al*., 2019; Kapil *et al*., 2017).

In Table (7) we note that the honey does not affect the small concentrations (1, 2 and 3) g on the stimulation of the composition of the roots were affected by a simple concentration of 4 g 0.5 was the production of roots significantly at the concentration of 6 g 2.5 and also at the concentration of 8 g, The concentration of 4 g / L of honey is the best concentration of the addition to the medium because it does not act as a hormone but acts as an antibiotic and as a resistance to contamination, while high concentrations stimulated the formation of roots in the concentrations (6 and 8) g/l.

In the results of the experiment we found that the best concentration of honey was 4 g / l in terms of being anti-contamination and it is not inhibitory to growth and does not affect the stimulation of callus production also had a role in enhancing the resistance to contamination after inocubation explants, this technique allows inocubation explants without passing in autoclave step (heat setrilization). This study is the first of its kind using honey as an antibiotic to get rid of contamination where it was not feasible to use antibiotics in the agricultural media, which are added to the medium after inocubating. The explants have an inhibitory effect on growth root, shoot even cells , stimulation, and difficulty in controlling antibacterials added as antibiotics. In addition, it is affected by high temperature as it is damaged by high heat and direct addition may be a source of contamination (Lefert and Waites, 1992; Kane, 2003).

Table 1 : Effect of honey on the percentage of contamination in inoculation without using an autoclave.

Percentage of contamination				
Honey g/l	**5day**	**10day**	**15day**	**20day**
0	100	100	100	100
1	0.0	30	80	100
2	0.0	20.6	40.6	80.2
3	0.0	10.3	20.3	30.3
4	0.0	6.0	8.0	8.0
6	0.0	2.0	4.0	4.0
8	0.0	0.0	2.0	2.0
L.S.D. 18.5				

Table 2 : Effect of honey on the percentage of contamination in medium with using the autoclave.

Percentage of contamination				
Honey g/l	**5day**	**10day**	**15day**	**20 day**
0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0

Table 3 : Effect of honey on the percentage of contamination in the medium using autoclave after inoculating the non-sterilized explants.

Percentage of contamination				
Honey g/l	**5day**	**10day**	**15day**	**20 day**
0	80.2	84.5	93.7	100
1	74.4	83.3	86.6	88.7
2	58.7	60.2	70.2	78.2
3	12.8	18.0	22.4	33.5
4	5.5	6.7	6.7	6.7
6	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
L.S.D. 25.8				

Table 4 : Effect of honey on the percentage of contamination in the medium using autoclave after inoculating the sterile explants

Percentage of contamination				
Honey g/l	**5day**	**10day**	**15day**	**20 day**
0	15.4	25.7	33.2	33.6
1	5.2	18.9	24.5	28.7
2	0.0	2.3	6.7	11.3
3	0.0	0.0	4.5	8.3
4	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
L.S.D 7.6				

Table 5 : The effect of adding different concentrations of honey in the medium on the soft weight of callus mg.

Honey g/l	**The soft weight of callus mg**
0	100
1	107
2	119
3	125
4	128
6	178
8	288
L.S.D 27.3	

Table 6 : Effect adding different concentrations of honey in the medium.

Honey g/l	seed status
0	Germination
1	Germination
2	Germination
3	Germination
4	Germination
6	Only bulge
8	Callus induction

Table 7 : The effect of adding honey at different concentrations in the medium on the number of roots growing from the shoot tip.

Honey g/l	Shoot tip
0	0
1	0
2	0
3	0
4	0.5
6	2.5
8	3.5
L.S.D. 1.39	

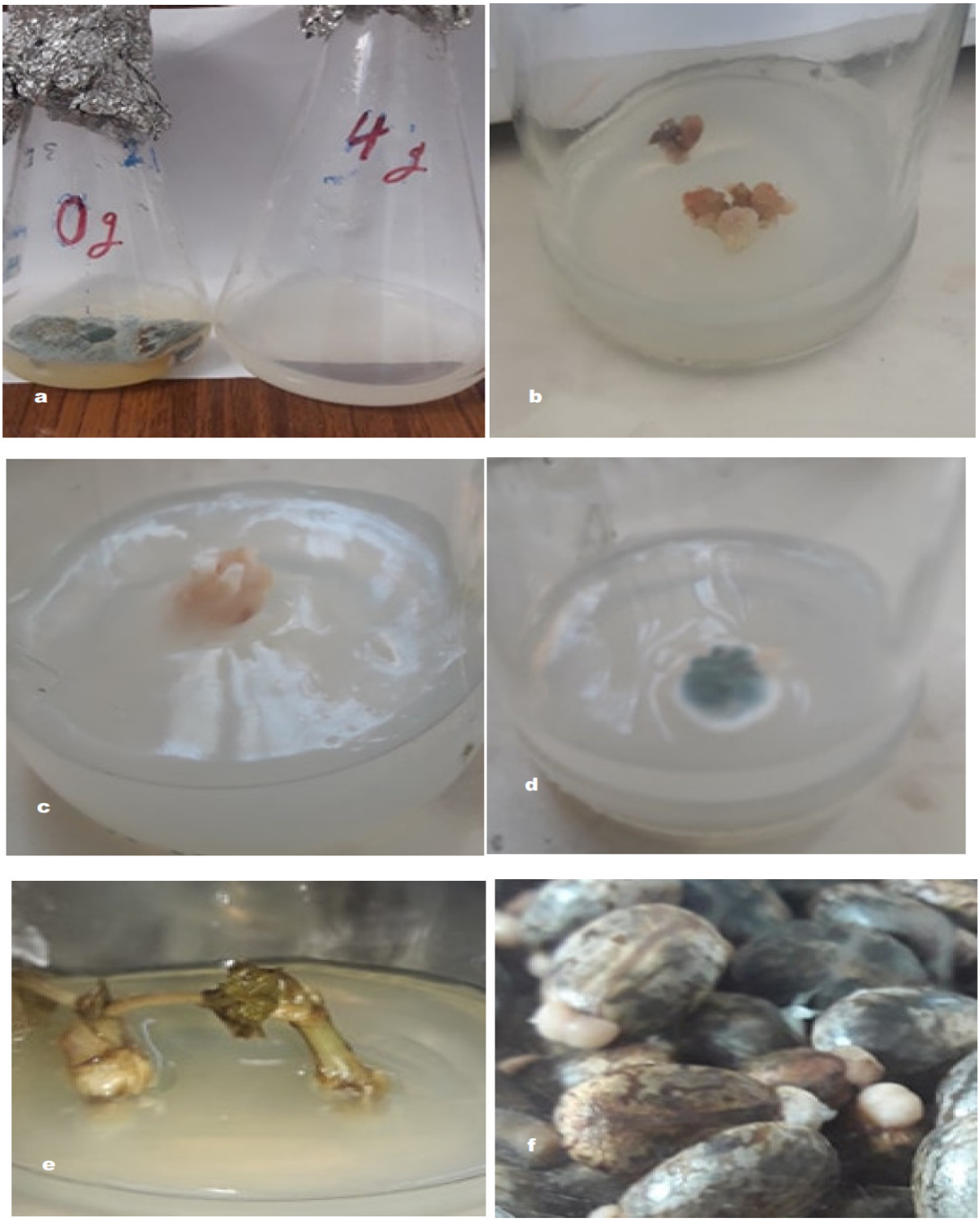

Fig 1. : (a) Effect of honey on the percentage of contamination in inoculation without using autoclave between control and 4mg/l honey; **(b)** The effect of adding honey in the medium on the soft weight of callus in 4 mg/l; **(c)** Explant not contamination after inoculation with honey; **(d)** Explant contamination after inoculation without honey; **(e)** The effect of honey on shoot under at 4 mg/l honey; **(f)** Seed fall in germination effected by a high concentration of honey.

Conclusion

The results showed that the addition of honey to medium has a significant effect in the conservation medium of contamination without use of autoclave at the concentrations (4, 6, 8) g / L of honey where there was no contamination during the incubation period 20 days**.**

the autoclaved medium did not contaminate at all the honey concentrations, the high concentrations effected in direction explants growth and tissues in the concentrations (6,8) g/L failed in seed germination, rooting shoot tip growth and stimulated callus growth while concentration did not appear 4g/L impact on the direction of growth in addition to being a medium resistant to contamination.

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