# Effect of plant extracts on inhibiting internal contaminants in vitro

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**Abstract:**
These study experiment were carried out in the Plant Tissue Culture Laboratory of the Department of Plant Production Technologies - Postgraduate Studies - Technical College of Mussaib - AL-Furat AL-Awsat Technical University for the period from 19/12/2022 to 4/15/2023.The present experiment aimed to determine the effect of plant extracts (cardamom, cloves, and haramel) at concentrations (0, 2, 4, 6) g. $L^{-1}$ of each in MS medium grown with saffron corms in tissue culture, to follow up the effect of extracts on growth results. The results of the triple intervention between the second, third, and fourth weeks with clove extract at a concentration 2 g. $L^{-1}$ was signification to other interventions in giving the highest value for the success of Implants remaining free of contamination, amounting to 25.00. There was also no significant effect of the plant extracts on the apparent characteristics of the saffron corm. The clove extract in the nutrient medium supplied with a concentration of (2 g.L-1) outperformed the rest of the plant extracts by giving it the highest average number of shoots, which reached 6.75 shoots, but it was not significant. Also, the prepared nutrient medium (6 g.L-1) of cardamom extract was not significantly superior in giving it the highest average shoot length of 9.50 cm, While the control treatment (0) gave the highest average number of roots reaching 63.8 roots, and the average length was 15.00 mm at a concentration of 4 g. $L^{-1}$ for cardamom extract.

**Key words:** Contaminant, Plant extracts, Clove extract

## 1.1 Introduction

The problem of contaminant is considered one of the major problems in tissue culture. Because of this problem, many measures are taken to sterilize media and cultures, and many sterilization methods and procedures can ensure the transfer of sterile explants to the sterile medium in a pathogen-free environment (Hussein and Khairallah, 2013). These complex processes increase the cost of plant tissue culture production and are also considered an influential factor in scientific experiments. In addition, sterilization materials have an impact on damage to explants and on the progress of production and scientific experiments. Although costly, these procedures do not protect many factors and laboratories from contamination, and some parts of plant are internal sources of contamination that cannot be eliminated through surface sterilization. It may appear on the medium after the plant has been growing for a few days. Adding antibiotics can effectively eliminate internal contamination in outplanted plants, although not all contaminants can be controlled with antibiotics (Tambarussi et al.,2015). Internal contaminants come from internal tissues of explants growing in a nutrient environment. Any internal contamination will make it difficult for the sterilization material to penetrate and reach and nutrient-rich environment supports the activity of these contaminants, it appears that contamination occurs on cultivated plant tissue and then becomes active throughout the nutrient environment when the appropriate temperatures for microbial activity are present in most cases, these contaminants are caused by bacteria or it could be a vascular fungus (Nasr al-Din et al.,2014). Plant extracts are used to inhibit the growth of many pathogenic microorganisms because they contain chemicals that effectively control the growth

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of these microorganisms, including harmala extracts, whose inhibitory effect is due to the compounds they contain, which include various types of flavonoids, Glycosides and others. It also contains four alkaloids important for suppressing contaminants Harmine, Harmaline, Peganine, and Vasicinone which constitute at least 5.9% of the dry weight of the seed (Hemateenejad et al., 2006) But these compounds have not been measured. Clove seeds are also highly effective in inhibiting Gram-negative and Gram-positive bacteria, due to the presence of Eugenol, which has antioxidant activity (Newal et al 1996). Hashim et al., (2013) also studied the inhibitory effect of phenolic extract on some bacterial isolates that contaminate food. The results showed a high inhibitory effect against all bacterial isolates under study and varied depending on the concentration and bacteria tested. Cardamom seeds also contain important compounds that have inhibitory effects on fungi and bacteria. Cardamom extract is used as an important agent in preventing viral growth (Duke et al., 2002).

**1.2 Materials and Methods**

The study experiments were carried out in the Plant Tissue Culture Laboratory of the Department of Plant Production Technologies - Postgraduate Studies - Technical College of Mussaib - AL-Furat AL-Awsat Technical University for the period from 19/12/2022 to 4/15/2023.

**1.2.1 Experimental evaluation and statistical analysis**

Observe all study trials using a completely randomized complete randomization design (CRD) and two factors for each trial knowing that the number of repetitions for each button is 10 defects. The results were analyzed using the Genstate statistical analysis program, and the averages of the coefficients were compared using the least significant difference (L.S.D.) test at the potential level of 0.05 (Al-Sahuki and Wahib, 1990).

**1.2.2 Source of explant**

We used saffron corms (*Crocus sativus* L.), which were brought from one of the approved offices in Basra Governorate at one year old and imported from Iran. The corms were kept at a temperature of 7°C for 21 days for the purpose of breaking dormancy and then starting to grow them in the laboratory. Growth regulators manufactured by CAISSON were also brought from Baghdad Governorate from a specialized office. Planting began in December.

**1.2.3 Preparation of plant extracts**

The aqueous extract was prepared by weighing 50 grams of dry powders of cardamom, cloves, and haramel separately, adding 1000 ml of distilled water to each weight in a glass beaker and leaving it for a full day at room temperature. Then the mixture was filtered using several layers of clean gauze to get rid of plankton. The plant extracts were then filtered using Whatman No. 1 filter papers .The extracts were dried in clean glass dishes at laboratory temperature for seven days until the weight was stable, then collected and stored in sterile tubes and placed in the refrigerator at 4°C until use (Khudair, 2021).

**1.2.4 Preparing the nutrient medium**

The well-known nutrient medium MS (Murashige and Skoog, 1962) was used in ready-made powder form from HI media Ltd. to grow saffron corms. I added 4.9 grams of the ready-made powder according to the manufacturer's recommendations to a 1-liter volumetric flask containing 700 ml distilled water, in addition to adding sucrose (30 g. $L^{-1}$) as a source of carbon and energy, except for the stage of corium formation. I used sucrose in several concentrations. Activated charcoal 0.75 g/L was added to all stages ingredients were mixed well in the Hot plate Magnetic Stirrer to dissolve them. Adding plant extracts that had been prepared previously in concentrations of (0, 2, 4, 6) g. $L^{-1}$ in the initiation stage, and fill the volume with distilled water to the mark, then the pH of the medium was adjusted to 5.70 using hydrochloric acid (HCl) or 0.1 m sodium

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hydroxide (NaOH) solution. Agar-Agar was added in an amount of 7 g. $L^{-1}$ to the medium, and place the medium in a microwave device for 5 minutes and heat it for the boiling point to dissolve it and homogenize the nutrient medium, then pour the medium directly into the culture tubes (glass screw vials) at a rate of 10 ml for each tube and close it with its own lid and then sterilize it in an autoclave at a temperature 121 mº and a pressure of 1.04 kg $cm^2$ for 20 minutes. After completion of sterilization, it was stored in a sterile place until planting. The plantlet were incubated in the growth room under incubation conditions at a temperature of 25± 2C 16 hours of light and 8 hours of darkness. The results were recorded after 21 days.

**1.4 Results & Discussion**

**1.4.1 The effect of plant extracts on inhibiting effectiveness of internal contaminants in saffron buds**

The results of statistical analysis in Table (1) show that treatment of second week was similar to treatment of third and fourth weeks by giving it highest rate of 18.75, and thus it was significantly superior to rest of treatments in preventing the growth of internal contaminants, while treatment of third week gave the lowest rate of 4.69. In the same table, the concentration (6 g. $L^{-1}$) outperformed the rest of the concentrations by giving it the highest average of 18.75. It is also noted that adding clove extract was significantly superior in preventing the activity of internal contaminants on explant grown inside nutrient medium, giving it highest rate of 18.75. The binary interaction between (contamination × concentration) gave the highest ratio in the second week of treatment, reaching 18.75 at a concentration of 4 g. $L^{-1}$, It didn't differ significantly from the rest of the treatments except with the fourth week treatment at the concentration of 2 g. $L^{-1}$, which had a success rate of 10.42 and the culture remained contamination-free. On the other hand, the binary interaction between (extract × concentration) had a significant effect. The intervention (cloves at concentrations 2, 4, 6 $g.L^{-1}$) respectively achieved highest success value in keeping saffron corms free from internal contamination, amounting to 25.00, and it was significantly superior to rest of the treatments, except for Harmal treatment at concentration of 6 gm. $L^{-1}$.The results of the triple interaction also indicate that there are significant differences between the study factors combined, as the interaction was achieved (The second, third, and fourth-weeks × cloves × 2, 4, 6 g. $L^{-1}$) respectively, the highest success value in keeping the implants free from internal contamination was 25.00 and did not differ significantly with the intervention (The third week × Harmal × concentrations 4, 6 g. $L^{-1}$) The lowest value during the interaction (fourth week × cardamom × concentration 2 g. $L^{-1}$) was 0.00. It was shown from the results of the same table that plant extracts inhibited the effectiveness of internal contaminants, and at different concentrations, they showed a significant effect in preventing the growth of internal contaminants, and the development and growth of the explant was better. The nutrient medium supplied with clove extract at a concentration (2 $g.L^{-1}$) outperformed other plant extracts in preventing the formation of internal contaminants in the nutrient medium and successfully kept the crops free of contamination. The reason for preventing the growth and activity of internal contaminants in the explant is that these plant extracts contain chemical compounds, such as Harmine, Harmaline, Peganine, and Vasicinone, which are contained in the Harmal plant. They have an effective effect in controlling the growth of these contaminants, and this is what was confirmed (Hemateenejad et al, 2006; Fazza, 2013: Hashim et al., 2013). Onywere et al. (2023) also confirmed that aquatic plant extracts of the mint plant have a high inhibitory activity in preventing the growth of bacterial and fungal organisms. Eugenol is also found in cloves, which has antioxidant activity and a major role in inhibiting Gram-negative and Gram-positive bacteria (Newal et al.,1996).

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**Table (1): Effect of plant extracts on the inhibition of internal contamination in vitro**

| Contamination | Plant extracts | Concentrations (g/L) | | | | Interaction between extracts × Contamination |
|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | |
| Second week | Cardamom | 0.00 | 6.25 | 12.50 | 12.50 | 7.81 |
| | Cloves | 0.00 | 25.00 | 25.00 | 25.00 | 18.75 |
| | Haramel | 0.00 | 12.50 | 18.75 | 18.75 | 12.50 |
| Third week | Cardamom | 0.00 | 6.25 | 12.50 | 12.50 | 7.81 |
| | Cloves | 0.00 | 25.00 | 25.00 | 25.00 | 18.75 |
| | Haramel | 0.00 | 12.50 | 18.75 | 18.75 | 12.50 |
| Fourth week | Cardamom | 0.00 | 0.00 | 6.25 | 12.50 | 4.69 |
| | Cloves | 0.00 | 25.00 | 25.00 | 25.00 | 18.75 |
| | Haramel | 0.00 | 6.25 | 12.50 | 18.75 | 9.38 |
| L.S.D. 0.05 | | Triple interaction: 12.949 | | | | 6.475 |
| Contamination | | | | | | |
| Interaction between Contamination × concentration | Second week | 0.00 | 14.58 | 18.75 | 18.75 | 13.02 |
| | Third week | 0.00 | 14.58 | 18.75 | 18.75 | 13.02 |
| | Fourth week | 0.00 | 10.42 | 14.58 | 18.75 | 10.94 |
| L.S.D. 0.05 | | Interaction between Contamination × concentration 7.476 | | | | 3.738 |
| Extracts | | | | | | |
| Interaction between extracts × concentrations | Cardamom | 0.00 | 4.17 | 10.42 | 12.50 | 6.77 |
| | Cloves | 0.00 | 25.00 | 25.00 | 25.00 | 18.75 |
| | Haramel | 0.00 | 10.42 | 16.67 | 18.75 | 11.46 |
| L.S.D. 0.05 | | Interaction between extracts × concentrations | | | | 3.738 |
| Concentration (g/L) | | 0.00 | 13.19 | 17.36 | 18.75 | |
| L.S.D. 0.05 | | 4.316 | | | | |

**1.4.2 Effect of plant extracts on phenotypic characteristics of saffron**

From the results in Table (2) it is clear that there was superiority, but it was non-significant the nutritional properties of saffron plants grown in nutrient medium containing plant extracts are in terms of number of shoots and length of shoots, number of root and root length, its growth better compared to vegetable characteristics that were grown in food media devoid of plant extracts. Vegetative characteristics were affected and their growth stopped due to the appearance of internal contaminations, which caused damage to explants. Clove extract in the nutrient medium at a concentration of (2 $g.L^{-1}$) had the highest average number of shoots, reaching 6.75 shoots, which was better than other plant extracts, but this was non-significant. In the same table, the prepared nutrient medium (6 $g.L^{-1}$) was non-significantly better than cardamom extract with an average shoot length of up to 9.50 cm. From the clear results in the table, it is clear that the plant extracts have non-effect on apparent characteristics of the saffron corm, and it has grown and developed well, and that the effect of the plant extracts was only in preventing the growth of contamination that cause damage to the explant and then the death of the explant. The

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reason for its effect on contaminant is that it contains chemicals that play a major role in inhibiting the activity of fungal and bacterial contaminations (Duke et al, 2002).

**Table (2): shows the phenotypic characteristics of saffron plants in vitro**

| Plant extracts | Concentration (g. $L^{-1}$) | Number of shoots | Length of shoots (cm) | Number of roots | Length of roots (mm) |
|---|---|---|---|---|---|
| **Cardamom** | 0 | 4.75 | 6.00 | 63.8 | 10.00 |
| | 2 | 3.00 | 5.75 | 56.0 | 10.25 |
| | 4 | 6.25 | 8.50 | 48.8 | 15.00 |
| | 6 | 3.00 | 9.50 | 38.8 | 12.50 |
| **Cloves** | 0 | 4.00 | 8.25 | 61.2 | 11.25 |
| | 2 | 6.75 | 7.50 | 60.8 | 13.75 |
| | 4 | 4.50 | 7.75 | 36.2 | 11.25 |
| | 6 | 5.25 | 6.50 | 51.0 | 7.50 |
| **Haramel** | 0 | 6.50 | 8.25 | 58.8 | 12.50 |
| | 2 | 4.25 | 7.25 | 46.2 | 15.00 |
| | 4 | 3.50 | 5.50 | 46.2 | 9.00 |
| | 6 | 4.25 | 6.50 | 55.0 | 8.75 |
| **L.S.D. 0.05 to Interaction** | | **3.775** | **4.068** | **21.87** | **8.905** |


**Figure 1: Figure (A) showing that the nutrient medium in which the plant part grows is free of internal contamination. (B) Explain the fungal and bacterial contamination that affected the explant. (C and D) show the growth of roots on a nutrient medium containing plant extracts**

**1.5 Conclusions**

The addition of plant extracts suppressed internal contaminants produced two weeks after planting and had no significant effect on growth trends.

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