Egypt. J. Agron. **Vol. 46**, No. 1 , pp: 179 - 190 (2024)

Egyptian Journal of Agronomy
http://agro.journals.ekb.eg/

Control of Microbial Contamination during The Micropropagation Process of Some Fruit Trees

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THE current article will review the efficient and recent technologies for controlling the microbial contaminants at plant tissue culture stages. Several sources can originate contamination in plant tissue culture such as mother plants, surface sterilization, and *in vitro* contamination by bacteria and fungi. Explant contamination results from donor plants as well as environmental factors such as surface sterilization, age, size, and source of the explants, as well as explants kept under stringent sanitary conditions. Many issues in the greenhouse come to an end if robust branches with functioning leaves are developed during the establishment stages. During the establishment stages, chemicals like H_2O_2, $AgNO_3$, $Ca(ClO_2)$, and $HgCl_2$ also showed good results for the surface sterilization of some fruit trees. Reducing contaminations can help explain this, and it also depends on the kind of tissue and the type of explant being utilized for micropropagation. Both Carbenicillin and Cefotaxine, when used alone or in combination, are the most effective antibiotics against bacterial contamination in plant tissue culture. Recently, nanomaterials as modern agriculture sector was applied to solve microbial contamination problems, such as using nanochitosan and nanosilver to control total microbes.

Keywords: Contamination; Sterilization; Nanomaterials; Nanochitosan and Nanosilver Micropropagation

Introduction

Micropropagation plants may become contaminated for several reasons. These can appear out of nowhere if surface sterilization is inadequate and bacteria that were hidden in explants, introduced during sub-culturing, or contaminated concurrently in cultures following a protracted development period (**Hesami et al., 2021**). Contaminants with microorganisms such as bacteria, fungi, yeasts, viruses mollicutes and rickettsias have been causing significant economic losses in plant tissue culture laboratories (**Al-Dasary et al., 2011**). Therefore, it is crucial to establish an efficient sterilization procedure. Because antibiotics affect plastids, mitochondria, and chlorophyll production, their application in managing plant pollutants is restricted (**Verhaegen et al., 2023**). Within three to four days of bud transfer to a sterile mineral salt (MS) medium, the base of the explants began to show signs of microorganism development (bacteria and/or fungi). The microorganisms present in the explants, poor aseptic procedures employed whilst working, and insufficient surface sterilization of the explants could have all contributed to this issue (**Mihaljević et al., 2013; Niedz and Bausher, 2022**).

For the complete eradication of germs, surface-sterilizing chemicals work better than mercuric chloride or sodium hypochlorite alone (**Omamor et al., 2007**). The favorable outcome of the combination of approaches in this experiment may have resulted from the $HgCl_2$ and NaOCl's sufficient synergistic effect on short-term microbe survival suppression; as a result, the cultivated explants were unaffected. **Hesami et al. (2017)** found the sterilization by commercial bleach (5% sodium hypochlorite) at 30% and mercuric chloride at 0.1 % or 15 % min duration were effective in sterilizing (Jojoba). The explants (apical shoot buds and nodal explants) were washed under running tap water then passed through surface sterilizing steps by immersion for 10 min in commercial bleach (Sodium hypochlorite (NaOCl)) supplemented with 2-3 drops of twenty tween, rinsed three times with sterile. Water and sterilized by $HgCl_2$ at 0.1% for 1 min surface sterilization of Fig trees (**Singh et al., 2010; Khalid et al., 2021**). Similarly, other

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Received 4/07/2024; Accepted 23/7/2024
DOI: 10.21608/agro.2024.300442.1457

researchers successfully sterilized several explants using mercuric chloride and sodium hypochlorite (**Osterc et al., 2004; Singh et al., 2011; Hassan et al., 2014**).

When plants are cultured in vitro, contamination with microbes such fungi, bacteria, viruses, and yeast is thought to be the main cause of losses (**Darwesh et al., 2019; Bhat et al., 2023**). **Telci et al. (2011)** reported that application periods of NaOCl below 3.75% and 15 min was more effective in increased seed-borne contamination and decreasing concentrations sterilized by 5.00% NaOCl concentration was more effective in dramatic decreases were observed at in all cases and deleterious effects on the embryo of the seeds. When NaOCl concentration increased to 5.00 from 3.75 % for 15 min application seed germination decreased to 65.18%; adding 3.75% NaOCl concentration for 15 min in to the culture medium were observed faster as compared with other used treatments of the seeds sterilized. During the *in vitro* establishment phase, the most material loss is caused by fungal contamination (**Darwesh et al., 2018)**. **Teixeira et al. (2006)** found that supplemented in culture medium 0.005 and 0.007% of active chlorine give a greater number of shoots and greater shoot length for *Eucalyptus pellita* but demonstrate the positive influence of low concentrations of NaOCl on morphological characteristics. Moreover, monosaccharides undergo heat degradation at high pressure during autoclaving because the process hydrolyzes sucrose into its monomers, glucose and fructose, as well as other products. This can negatively affect the growth. Active chlorine concentrations ranging from 0.001 to 0.003% promoted the *in vitro* development of *E. benthamii* nodal segments, and the results were comparable to those obtained using the conventional produced culture medium. Thus, by using lower doses of sodium hypochlorite, nodal segment establishment can occur in vitro without the necessity for autoclaving. This significantly cuts down on the time required to prepare the culture media and avoids the high energy expenses associated with autoclaving. To validate the feasibility of sodium hypochlorite as a substitute for conventional cultivation, more research is necessary to determine how this process affects other stages of micropropagation, such as bud multiplication, elongation, and shoot rooting. Testing other compounds is also necessary, keeping in mind that many of these substances have the potential to be harmful (**Taha and Hassan, 2016**). **Parasharami et al. (2014)** the best face sterilization (80%) for fruit of *F. religiosa* was obtained in when the explants were sterilized by 2% sodium hypochlorite for 30 min. In review, the following contamination suggestions are offered to assist in contamination control in plant tissue cultures: -

1- Maintain a clean tissue culture facility and utilize established aseptic procedures.
2- Utilized the healthiest explant source plant available and grow these for 2-3 weeks prior to explant removal under cool, dry conditions that will also promote plant growth.
3- Remove extraneous plant tissue and any residue from the explant before disinfestations.
4- Utilize as small an explant as possible.
5- Index resultant cultures for microorganisms to develop contamination-free lines.
6- Where needed, do assays of resultant cultures for virus or virus like organisms.

The biggest issue with micropropagation, despite the fact that a high number of plants can be produced, is contamination. A wide range of microorganisms (bacteria, filamentous fungi, yeasts, viroids and viruses) and micro-arthropods (mites and thrips) have been can originate contamination in the tissue cultures during manipulations contaminants in the laboratory, by micro-arthropod vectors Contaminants may be introduced with the explant (**Permadi et al., 2023**). Modern agriculture biotechnology deals to apply new disinfectant materials compatible with tissue culture techniques and did not has negative effects on plant propagation. From such materials, extracted bioactive compounds with microbial and plant nature and new nanomaterials with biological activity (**Mittal et al., 2020; Eweys et al., 2022; Darwesh and Elshahawy, 2023; Darwesh et al., 2023**). Even though nanoparticles have a wide range of prospective applications, particularly in the environmental sector, there are certain important considerations that need to be made. In order to prevent the excessive and improper usage of nanocomposites from compromising human health, these warnings must be taken into scientific consideration without being overstated or denigrated. Furthermore, in order to prevent humans from losing out on the chance to benefit from such special nutrients, we shouldn't let our fears prevent us from using nanocomposites in a safe and sensible manner. It is important to consider the benefits and drawbacks of using nanocomposite applications as well as potential future applications (particularly in the environmental field) in order to ascertain the regulations and guidelines that must be followed when working with such a wide variety of substances. A lot of studies proposed that the NPs could be easily entered into human body due to their nanoscale dimension is similar size to typical cellular components (**Part *et al.*, 2015**). The challenge of assessing and classifying methods for nanowastes sufficiently is carefully associated with the methods adopted in founding the NM toxicity. It is suggested that studies on hazard assessment

should consider actual nanowaste watercourses. These studies should be planned to income into account all the related biotic and abiotic factors in order to offer the most accurate threat of the nanowastes. The best data should be resulted to explain the potential carrier ability of NMs to other environmental pollutants, whether the detected toxicity is due to separate NMs or aggregated form and how actual environmental factors impacts nanowastes risk.

Mother plants

Because the plants are cultured in a contained container, *in vitro* propagated plants frequently have aberrant physiological conditions. When field-grown plants are cultivated in a contained environment, such as beneath plastic film, these anomalies also appear. The saturation of the container environment with water vapor is most likely the primary cause of this anomaly. In the worst-case scenario, those physiological abnormalities might cause the plants to die entirely; following a stage where the material seems vitrified, the plants turn necrotic. The tissues glister as they were once transparent and bursting with water. When the illness is first developing, healthy branches appear to have normal-looking leaves. However, looks can be deceiving; in fact, those leaves frequently lack a wax covering and contain non-functional stomata. One of the main issues in the greenhouse is transferring explants from the culture container. Stated differently, the last phase in vitro and the initial phases in vivo are critical. Numerous issues in the greenhouse can be resolved if robust branches with functioning leaves are created during the acclimation stages (**Yadav et al., 2023**).

The mother plants need to be in good nutritional and phytosanitary condition, the contamination of *in vitro* propagated plants during isolation spraying systemic agent's sudations such as Terramycin (oxytetracycline) or benomy L (Ben late) with streptomycin sulfate is effective when compared with non–systemic fungicides containing different active principles. These treatments need to adhere to a strict chronogram, and the explants should be picked up no later than 24 to 48 h following the last fig tree (*Ficus carica* L.) spraying. The preservation of the mother plants in the greenhouse makes it possible to regulate and alter the temperature, light intensity, and photoperiod, all of which encourage the growth of new branches. Regardless of the season, fungicides, bactericides, and insecticides are useful and practical treatments for greenhouses. The newly developed brunches, immediately recovered the pruned branches should be covered with plastic bags (**Bonga, 1982**). In addition, culturing of protoplast KAO and Michayluk medium supplemented with 3.0 mg L^{-1} NAA and 0.2 mg L^{-1} BAP as well as the combination of antibiotic (0.4 mg L^{-1} Ampicillin + 0.1 g L^{-1} gentamycin + 0.1 g L^{-1} tetracycline) and using protoplast density at the rate of 2.5 x 10^4 induced the best protoplast viability and development of pineapple explants (**Ali et al., 2021**). The mother plant horticultural trails should be selected for tissue culture. The mother plant can be selected by phenotype or source of elite seed. However, most of these symptomless plants are preventing bacterial and fungal spores causing hindrance in their culture initiation. However, glass house-grown plants have been shown to give better response owing to low Phenolic exudation in medium and low fungal and microbial contaminants (**Ebile et al., 2022**).

The elements listed below were examined as ways to prevent contamination in plant tissue cultures. There are three main tissues, during sub-culturing and reducing microbial contamination in the culture at the stage of proliferation and rooting prevents the introduction of microorganisms with the initial plant and prevents their introduction from the environment. Preventing bacterial contamination *in vitro* is the most effective way to prevent the elimination of bacteria from the initial plant explant that is introduced into the culture. The most effective way to minimize contaminations is to employ donor plant explants under a rigorous hygienic protocol, effectively sterilize the original explants, and reduce the size of the initial explants to apical meristems. There are various sterilizations according to the species of plants. The surface contamination depends on the growth environment, plant material, age, and part of the plant used for micro-propagation (**Hasnain et al., 2023**). Several plant and environmental conditions, including plant species, age, the source of the explants, and the current weather, might lead to contamination of the transplants (**Hussain et al., 2012**).

Surface sterilization of explants

Two sources can originate Contamination in tissue culture, each through the carrying of microorganisms presents on the explant surface or in the tissue itself (entophytic microorganisms). While most bacteria should be eradicated in meristem cultures, depending on the size of the meristem, the infection spreads to the cultures in leaf, petiole, and stem explants. Washed meristem culture with soap and tap water inside the laminar flow hood, the plant material was soaked in $HgCl_2$ and NaOCl is the two most widely employed surface sterilization processes that drastically reduced the microbial infection. Chemical solutions such as sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide and silver nitrate were effective in the surface sterilization of explants, the most effective treatment against surface sterilization of explants in the laboratories used sodium or calcium hypochlorite (**Hasnain et al., 2023**). Hydrogen

peroxide (H_2O_2) sterilization proved to be efficacious even in cases where there was a high percentage of contamination and a low percentage of explant survival. **Onwubiko et al. (2013)** discovered that hydrogen peroxide sterilization achieved 50% surface sterilization, making it the most effective treatment for explant surface sterilization. The explant flower buds of Jojoba (Male and Female explants) were sterilized using a solution of commercial bleach (66cloro × 5.25% available chlorine) at two concentrations of 10 and 20% for 15 min. The other sterilizing agent used was mercuric chloride ($HgCl_2$) at 0.05 and 0.1% for l0 min (**Gu et al., 2022**). **Brondani et al., (2013)** found that sterilized shoot tips (2 mm) by immersing in 70% ethanol for a minute and then by dipping in 0.2 solution of mercuric chloride ($HgCl_2$) for 3 min, giving the highest sterilizing of apple (*Malus domestica*). **Dutra *et al.* (2009)** found that sterilization by 16.6 g/L DICA (sodium dichloroisocyanurate) for 15 min was effective in protection of cherry plants. While, El **Hammady *et al*., (2005)** indicated that dipping explants in 10% of sodium hypochlorite for 8 min was effective in sterilizing Almond shoots (*Prunus dulcis Mill*).

Washed shoot tip of Sultani and Conadria fig cultivars with tap water then passed through surface sterilizing by immersion for 10 min in commercial bleach (Sodium hypochlorite) with 2-3 drops of twenty tween, rinsed three times with sterilized distilled water, and later immersed in HgCl for 1 min (**Darwesh et al., 2021**). Additionally, **Wolella (2017)** found that, for nearly all exposure time levels, contamination decreased when sodium hypochlorite concentration rose from 1 to 3% and vice versa when $HgCl_2$ concentration increased from 0.05 to 0.2%. When explants were disinfected with 1% NaOCl for 20 min in combination with 0.2% $HgCl_2$ for 5 min, or with 2% NaOCl for 15 min in combination with 0.1% $HgCl_2$ for 5 min, there were no discernible changes in low contamination and minimum explant death. When explants were cleaned with 2% NaOCl for 15 min and 0.1% $HgCl_2$ for 7 min, the highest significant survival value (97%) was observed (**Fig. 1**).

Fig. 1. The best surviving and healthy explants were obtained during surface sterilization of axillary buds using 2% NaOCl for 15 min and 0.1% $HgCl_2$ for 7 min (Wolella, 2017)

Chemicals sterilization of tissue culture materials should be effective, cheap, available, and non-toxic. A chemical that is strong enough to prevent the growth of disease-causing germs, even in little amounts, is called an effective chemical (or sterilant) (**Lateef et al., 2021**). **Pasqual and Ferreira (2007)** discovered that several chemicals, including ethanol and chlorine-based ones like calcium and sodium hypochlorite (0.5–2%), had germicidal properties and could be employed for explant sterilization. To improve the contact between the tissues and the chlorine solutions, a spreader-sticker surfactant, such as Tween 20 (1 to 2 drops 100 mL^{-1}), is typically employed to promote the tissue penetration capabilities of the solutions. Since ethanol at larger concentrations is less efficient and can quickly dry the tissues, it is often used for brief periods (between one and two seconds). In addition to its germicidal properties, ethanol is a surfactant and is used in the process of surface sterilization. Following sterilization, the explants are put in a nutrient medium and cleaned three to five times using distilled or deionized autoclaved water (*Ficus carica* L). In this regard, **Amiri et al., (2013)** found that sterilizing both rootstock shoot tips with sodium hypochlorite (Clorox solution 10, 15, and 20% for 15 or 20 min) had maintained the highest survival percentages significantly whereas, sterilizing freedom shoot tips their survival. Sterilized by 10% sodium hypochlorite, enhanced contamination control. Also, the use of Cefotaxime Antibiotic (400 mg L^{-1}) in comparison with a combination of Carbenicillin and Streptomycin antibiotics (each 200 mg L^{-1}) is more effective in contamination control of Mariana (*Prunus mariana*) rootstocks (**Grzebelus and Skop, 2014**). According to **Table (1)**, sterilizing agents such as sodium hypochlorite, calcium hypochlorite, sodium dichloroisocyanurate, mercuric chloride, silver nitrate, and hydrogen peroxide were found to be more toxic when their concentration and exposure time was varied, which resulted in the sterilization of 'Oblačinska' sour cherry buds. The explants (axillary buds and apical shoot buds) were washed under running tap water and then passed through surface sterilizing steps by immersion for different times exposure in commercial bleach supplemented with 2-3 drops of twenty tween washed three times with sterile distilled water. The results indicated that sterilization with $AgNO_3$ at a concentration of 1 % for 20 min was the best for controlling the effect of surface sterilization (**Mourad et al., 2019**).

TABLE 1. The common protocol of sterilizing agents at different concentrations with varying time for sterilizing axillary buds

Sterilizing agent	Concentrations % (w/v)	Time of exposure (min)
Sodium hypochlorite (NaOCl)	1	20
Sodium hypochlorite (NaOCl)	2	15
Sodium hypochlorite (NaOCl)	3	10
Calcium hypochlorite ($Ca(ClO)_2$)	1	30
Calcium hypochlorite ($Ca(ClO)_2$)	3	15
Calcium hypochlorite ($Ca(ClO)_2$)	5	5
Sodium dichloroisocyanurate (DICA)	2	20
Sodium dichloroisocyanurate (DICA)	1.5	15
Sodium dichloroisocyanurate (DICA)	1	10
Mercuric (II) chloride ($HgCl_2$)	0.1	10
Mercuric (II) chloride ($HgCl_2$)	0.5	5
Mercuric (II) chloride ($HgCl_2$)	1	2
Silver nitrate ($AgNO_3$)	1	20
Silver nitrate ($AgNO_3$)	1	10
Silver nitrate ($AgNO_3$)	1	5
Hydrogen peroxide (H_2O_2)	10	15
Hydrogen peroxide (H_2O_2)	20	10
Hydrogen peroxide (H_2O_2)	30	5

Sterilization with 0.1 % $HgCl_2$ for 7 min was found to be optimum for leaf explants of Poona fig. though 0.2 % for I min give highest sterilization but the explant establishment percent was decreased. A decrease in the concentration of disinfectant and duration of treatment resulted in a high percentage of contamination, while the increase in concentration, led to browning of shoot tip, higher $HgCl_2$ concentrations (0.2 – 0.4), and 4% NaOCl proved to be more toxic leading to browning and death of leaf explants (**Ahmadpoor et al., 2022**). The best surface sterilization was obtained when the explants were sterilized using 10–30 % of Clorox solution with two drops of tween 20 for 15-20 min on Jojoba (**Fig. 2**).

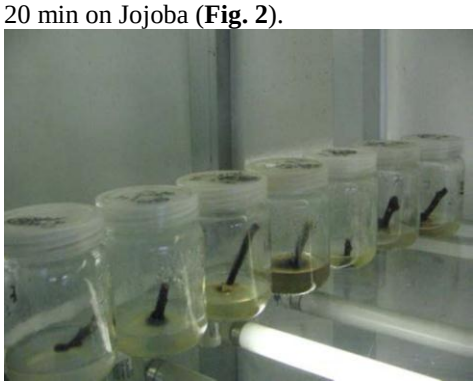

Fig. 2. Different surface sterilization of Jojoba plants

It was found that various concentrations of Sodium hypochlorite had a significant effect on seed germination, but different times of immersion did not have any significant impact on seed germination. The lowest rate of contamination (0%) was obtained with the treatments containing 20% Sodium hypochlorite at 10 and 15 min immersion and 25% Sodium hypochlorite at 5, 10, and 15 min immersion (**Fig. 3**). Treatments including 10% Sodium hypochlorite at 5 and 10 min immersion resulted in the highest seed germination (63.33%) and high percent of contamination except sterilized with10% Sodium hypochlorite at 15 min immersion (**Mourad et al., 2019; Abdelhameed et al., 2020**).

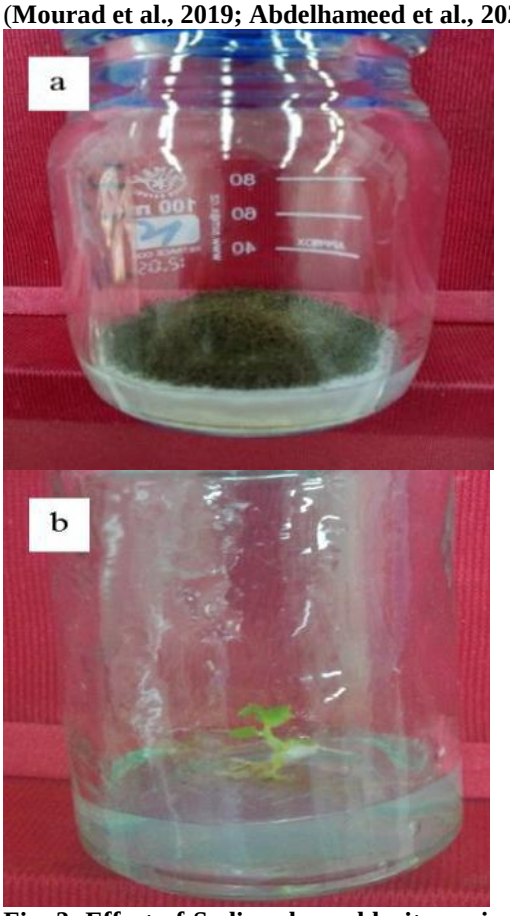

Fig. 3. Effect of Sodium hypochlorite on *in vitro* contamination: (a) infected medium, (b) clean medium

Control of bacterial contamination

Numerous species-specific microbes, including a broad range of bacteria, can contaminate plant tissue cultures. Bacterial infection can slow down a plant's pace of growth, prevent it from roots, or even kill it. Any step of the tissue culturing procedure can result in contaminated tissue cultures (**Al-Dasary et al., 2011**). The number of shoots in healthy cultures cultivated on medium enriched with Meta-Topolin alone was actually lower than in cultures grown on 500 mg L^{-1} carbenicillin. It has been observed that carbenicillin inhibits the formation of calluses in Clematis, Delphinium, Hosta, Iris, and Photinia, but increases callus formation in Anthirrhinum majus and Malus.

Because the pH, temperature, sucrose concentration, and salt concentration are not ideal for bacterial development during micropropagation, bacterial contamination may go unnoticed (**Izarra et al., 2020**). Endogenous bacteria are the hardest to control since they don't show any signs in the contaminated culture. Animal vectors or inadequate aseptic handling procedures can lead to the contamination of plant tissue cultures with a wide range of pathogens (**Hegazy et al., 2024a**).

Bacterial contaminants that were present in modest quantities can actively grow and harm plant cultures when the culture conditions are altered. After growing for three weeks on a medium enriched with carbenicillin, the growth of the bacteria was reduced but not totally stopped. There was also a significant amount of high-quality shoot formation (**Hegazy et al., 2024b**). The shoots were clear of both contaminating bacteria after nine weeks of development on medium treated with 40% Carbenicillin. 37% of the shoots on medium supplemented with Cefotaxine were clear of both contaminating bacteria after three weeks of growth. However, compared to shoots grown with carbenicillin, those grown with cefotaxine tended to be shorter and have yellower leaves (**Gabryszewska, 2005**). Many people continue to hold the belief that al1 bacterial contaminations in tissue cultures have an outside origin and are the result of improper sanitary practices. Nonetheless, a wealth of evidence indicates that endogenous microorganisms are also contributing to this issue (**Weiskirchen et al., 2023**).

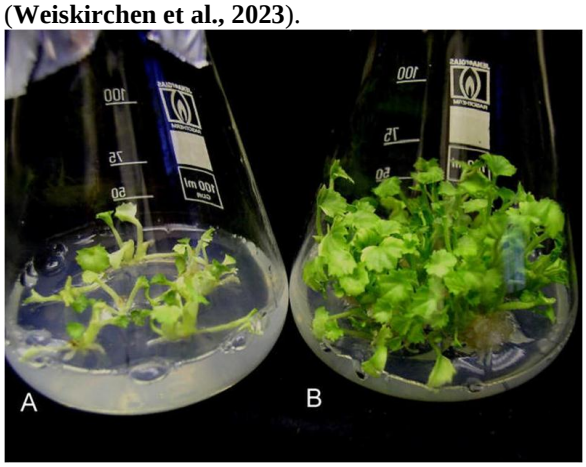

Fig. 4. The plant shoots after 3 weeks of growth on the medium containing cefotaxime (A) and carbenicillin (B)

Control of Fungi Contamination

In tissue culture laboratories, a variety of compounds with fungicides can be employed to stop or manage fungal contamination. The systemic fungicide benomyl worked similarly to cytokinin. Benomyl was added to the culture medium in different doses (10, 25, 50, 100, and 250 µg g^{-1}). At low doses (10 and 50 µg g^{-1}) benomyl encouraged effective development, but at high levels (100 and 250 µg g^{-1}) benomyl negatively affected shoot and root formation and also caused an abnormally short and thick shoot development. The main diseases for lilies are the fungus *Fusarium oxysporum* and *C. radicola* (**Chastagner et al., 2018**). Fungal contamination issues are often the less serious ones because the right sterilizing agent may usually resolve them. Fungi are saprophytic soil organisms that are common plant diseases. Numerous fungal species are related to plant tissues, and they typically contaminate those tissues. The primary culprits are *Aspergillus*, *Candida*, *Microsporum*, and *Phialophora* species (**Ahmadikia et al., 2021**). Microbial contamination results in material, time, and effort waste as well as significant financial losses. In most tissue culture laboratories worldwide, total losses resulting from microbial contamination range from 3 to 15%; the main sources of contamination are bacterial, fungal, and yeast agents (**Chastagner et al., 2018**).

According to **AL-Kaby (2004),** sterilization with 0.5-1 g L^{-1} of both fungicides carbendaz and score was successful in reducing the overall percentage of contamination without having any negative effects on growth. Fungal contamination, which includes both fungi and yeasts, caused damage to tissue cultures in a number of ways, including increased turbidity, altered medium acidity, and cell death. **Hamed and Abass (2006)** found that numerous date palm tissue cultures infected with fungal species, such as *Aspergillus niger* and *Alternaria alternata*, exhibited a significant degree of hydrolytic activity of phenol oxidase and cellulase enzymes *in vitro*. *Aspergillus nigar* demonstrated the highest amount of Phenol Oxidase activity in vitro, with an activity zone of a total of 18.60 mm. Meanwhile, *Alternaria alternata*, a filamentous fungus, demonstrated the highest degree of cellulase enzyme activity, with an extracellular activity zone measuring 1.50 mm. The breakdown and browning of infected tissue brought on by the release of chemicals into the medium, such as toxins and degradation enzymes (Cellulase, Phenol Oxidase, and others), are the main varied consequences of microbial contamination on date palm tissue culture. Furthermore, **Abass et al.'s study (2007)** showed how Ben late fungicide effectively prevented the growth of contaminated fungus *in vitro*. Ben late treatment reduced the proportion of contamination in date palm tissue culture from 25% in the control treatment to 1.65%. With frequencies of 27, 25, and 18%, respectively, fungi, *Aspergillus niger*, *Penicillium* spp., and *Alternaria alternata* were the most frequently isolated contaminants. A recent study conducted by **Al Mayahi et al (2010)** concluded that coating the Le Conte fruits with *Moringa* oil recorded a successful reduction in fruit weight loss, decay percentages, improving fruit quality and extending

storage fruit period and shelf life, also safe on environmental and human health (**Saleh et al., 2019**). Also, the highest value was achieved from the combined treatment gave of fruit TSS% and total soluble sugars while decreasing TA% weight loss % and fruit decay % during cold storage for 40 days. **Altan *et al.* (2010)** found that sterilizing by Benomyl (100 mg dm^{-3}) + Nystatin (100 mg dm^{-3}) of *Lilium* was an effective treatment against fungal contaminations.

Nanomaterials for surface disinfection of explants

Even before the propagation mechanism is started, microbial infection might destroy the entire process and efficiency. Removal of microbial contamination for *in vitro* culture of valerian officinal is when single node explants were surface disinfected with 70% ETOH for 180 min, then 100 mg L^{-1} of AgNPs for 180 min **(Altan *et al.*, 2010)**. However, the effect of AgNPs on the medium was found to be best for sufficiently controlled internal contaminants of olive "Mission" explants treated with 4 mg L^{-1} AgNPs (**Darwesh et al., 2021b**). The use of AgNPs in plant tissue culture was examined, it was observing that used of AgNPs at 500 mg mL^{-1} under reduced pressure (300 mg Hg) for 5 min, a biological contamination in plant tissue culture has successfully controlled the microbial contamination. In addition, inhibited microbial growth was obtained by adding AgNPs at rate of 50 mg L^{-1} to Murashige and skoog medium (**Matter et al., 2021**). Recent study has shown that surface disinfection of explants with 1.5% NaOCl for l0 min and AgNPs at rate of 200 mg L^{-1} for 15 min significantly reduces microbial contamination in various plants (**Darwesh and Matter, 2021**). The addition of NS and TiO_2 to tissue culture media for removing microorganisms *in vitro* culture and the next plants can growth very well. It is also reported that bacterial and fungal contamination decreased with 60 mg L^{-1} TiO_2NPs. Removal of microbial contamination for *in vitro* culture shoot buds of tobacco and Potato on MS medium had AgNPs or TiO_2NPs. The addition of ZnNPs and ZnO particles at different concentrations (40, 100, and 200 mg L^{-1}) can eliminate bacterial contamination of banana *in vitro* cultures (**Pathak et al., 2023**).

Using nanomaterials, which are extremely small substances with special properties that range from 1 to 100 nm, is one of the newest ideas. Tissue culture media can be utilized with a variety of nanomaterials, metals, and metal oxides, such as silicon (Si), magnesium oxide (MgO), nickel (Ni), zinc (ZnO), copper (Cu), silver (Ag), gold (Au), and nickel (Ni). Nowadays, nanotechnology permeates every facet of life, including food, medicine, and the environment. They come in a variety of sizes and shapes, including powder, particles, tubes, films, and clusters. The ability of nanoparticles (NPs) to eradicate various bacteria is its most well-known function. The most widely employed nanomaterials across several industries, particularly in agriculture, are silver nanoparticles (**El-Shanshoury et al., 2020**).

The obtained results by **Darwesh et al., (2023b)** suggested that regarding the variation in the produced nanoparticles' inhibitory capacity on *in vitro* microbial contamination, a significant difference was seen among the different types and concentrations of nanoparticles (**Fig. 5**). Silver nanoparticles at a concentration of 10 mg L^{-1} showed the lowest value of microbial contamination in the tissue culture media when compared to the negative control plates. The highest value was obtained by selenium nanoparticles, followed by chitosan nanoparticles. The outcomes suggested that using nanoparticles as antibacterial agents in tissue culture medium without sterilization is feasible. The ability of nanoparticle treatments to limit the growth of bacteria in vitro supports earlier research on the antimicrobial efficacy of the tested nanoparticles (**Yılmaz et al., 2023**). The results show that silver nanoparticles (AgNPs) have the greatest potential for eliminating microbial contamination in the culture medium. AgNPs recorded the lowest contamination percentage, which is statistically equivalent to media sterilized by autoclaving (**Fig. 5**). The remarkable antibacterial activity of AgNPs may be due to the substantial toxicity of silver to a wide range of microorganisms. Furthermore, the produced silver nanoparticles' small particle size (5–15 nm) is crucial for interactions and binding of silver with cell membrane proteins, which causes cell death (**Bruna et al., 2021**). When compared to selenium and the control treatment, chitosan nanoparticles (ChNPs) shown comparatively significant antibacterial activity; the antimicrobial efficacy of ChNPs against many plant pathogens has been previously described. DNA replication may be inhibited and the cell membrane permeability is affected by chitosan NP. Comparing SeNPs to AgNPs and ChNPs, our data revealed that SeNPs had less antibacterial activity; this reduced toxicity may be caused by SeNPs' negative charge, which causes a relative aversion of the bacterial membrane (**El-Shanshoury et al., 2020**). Furthermore, it might take larger SeNP concentrations to have a noticeable impact on microbiological contamination. According to a prior study, the minimal dose required to inhibit *S. aureus* and *E. coli* is 50 mg L^{-1}.

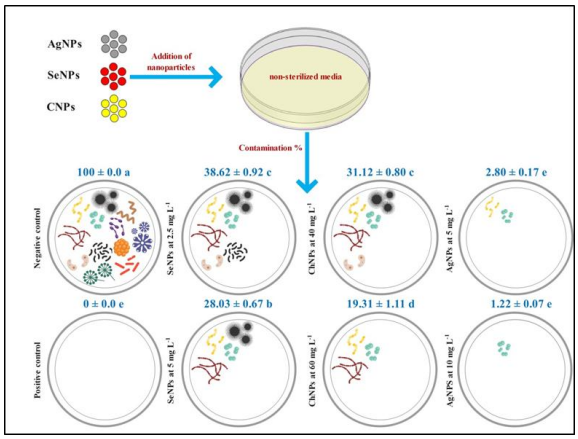

Fig. 5. Flowchart about the effect of silver, selenium and chitosan nanoparticles on *in vitro* microbial contamination; AgNPs, silver nanoparticles; SeNPs, selenium; ChNPs, chitosan nanoparticles; negative control (non-sterilized medium); positive control (autoclaved medium)

Several systems can be used for the green manufacturing of nanoparticles. Green synthesis techniques produce nanomaterials by using natural biological systems or eco-friendly processes. These techniques seek to lessen the high energy input and hazardous chemical usage connected with conventional synthesis techniques. Examples of natural sources utilised in green synthesis are enzymes, vitamins, biodegradable polymers, and microwave-assisted techniques; bio-based techniques include plant extracts and microorganisms **(Ijaz et al., 2020; Nair et al., 2022).** Enzymes are useful for the creation of some nanoparticles because of their clearly defined structure and readily available purity. They stabilize and functionalize the produced nanoparticles to a great extent. Enzymes play a dual role in the creation of metallic nanoparticles, serving as both reducing and capping agents. Occasionally, the amino acids that the enzymes produce serve as the process's stabilizing and reducing agents. Because free and exposed S-H groups are present, enzymes such as ECORI enzymes are productive. The enzyme pureα-amylase is used to create gold nanoparticles, and in the AuNP-α-amylase combination, the enzyme still has its catalytic activity. Enzymes such as cellulose, nitrate reductase, sulphite reductase, ligninase, and others are also utilized in the production of various nanoparticles **(Nair et al., 2022)**. Microalgae are recognized as practical and efficient "bionanofactories" for the production of metallic nanoparticles. Their ability to do so is dependent upon the extracellular and intracellular biomolecules (enzymes and functional groups that act as reducing agents to convert metals into nanoform) that they produce during growth. Techniques for synthesizing nanoparticles from freshwater and marine algae have been reported. Additionally, microalgae provide large-scale synthesis opportunities without the need for safety precautions, together with the advantages of autotrophic growth and rapid multiplication **(Punia et al., 2023; Abdel-Gawad et al., 2024)**. As a result, reliable, non-toxic, high-yielding, and ecologically sustainable synthesis processes are required to meet the growing demand for metallic NPs like silver nanoparticles. As a result, the biological green synthesis approach can provide a compelling alternative to physicochemical synthesis methods. After synthesis is complete, precise and targeted particle characterization is required since the physicochemical characteristics of the particle have a major influence on their biological properties. Before being used, the produced nanoparticles must be dynamically described in order to overcome the security concern and utilise nanomaterials to the fullest extent possible for the health care industry, human well-being, and the development of nanomedicines, among other applications **(Abbar et al., 2022)**.

Conflict of Interest: No conflict of interest is associated with the data associated with this work.
Data Availability Statement: The datasets used during the current study are available from the corresponding author upon reasonable request.
Competing interests: The authors declare that they have no competing interests.
Funding: No external funding was received

References

Abass, M.H. V.A.M. Al Badi, and Alkeby, A.M.S. (2007). The efficiency of hema leaves extracts and some fungicides to reduce the fungal contamination of date palm (phoenix daclyliferal) tissue culture. Iraqi J. Biotech. 6 (2): 1- 40.

Abbar, J.C., Swetha, G.A., Meti, M.D. and Byadagi, K.S., (2022). Environmental applications of green engineered silver nanoparticles. In Phytonanotechnology (pp. 199-225). Singapore: Springer Nature Singapore.

Abdel-Gawad, H., Matter, I.A., Abo-Alkasem, M.I., Al-Balakocy, N.G., Darwesh, O.M. (2024). Phyco-biosynthesis of Chlorella-CuO-NPs and its Immobilization on Polyester/Cotton Blended Textile Waste Activated by Cellulase Enzymes for Application as Wastewater Disinfection Filter. Egyptian Journal of Chemistry, 67(7): 609-621.

Abdelhameed, R.M. Darwesh, O.M. El-Shahat, M. (2020). Synthesis of arylidene hydrazinylpyrido[2,3-d]pyrimidin-4-ones as potent anti-microbial agents. Heliyon, 6: e04956, https://doi.org/10.1016/j.heliyon.2020.e04956.

Ahmadikia K, Aghaei Gharehbolagh S, Fallah B, Naeimi Eshkaleti M, Malekifar P, Rahsepar S, Getso MI, Sharma S, Mahmoudi S. (2021). Distribution, Prevalence, and Causative Agents of Fungal Keratitis: A Systematic Review and Meta-Analysis (1990 to 2020). Front Cell Infect Microbiol. 11: 698780. https://doi.org/10.3389/fcimb.2021.698780.

Ahmadpoor, F, Zare, N, Asghari, R, Sheikhzadeh, P. (2022). Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in vitro cultures Melia azedarach L. AMB Express. 12(1): 3. https://doi.org/10.1186/s13568-022-01343-8.

Al-Dasary, N.H., Al-Mussawi, M.A., Al-Taha, H.A. (2011). Isolation and identification of bacterial types that cause contamination of date palm (*Phoenix dactylifera*). callus and studying the inhibition activates of some plant extracts and antibiotics, Basra. J. Date Palm Res, 10 (1): 68-81.

Ali, S.S., Darwesh, O.M., Kornaros, M., Al-Tohamy, R., Manni, A., El-Shanshoury, A. R., Metwally, M. A., Elsamahy, T., Sun, J. (2021). Nano-biofertilizers: Synthesis, advantages, and applications. Biofertilizers: Advances in Bio-inoculants, Woodhead Publishing, Elsevier Inc., ISBN: 978-0-12-823030-5, 359- 370, https://doi.org/10.1016/B978-0-12-821667-5.00007-5.

Al-kaby, A.M.S. (2004). The effect of some antibiotics and fungicides on the growth of Embryogenic callus of date palm phoenix dactylifera L. Basra. J. Date palm Res. 3(1/2) 97-110.

Al-Mayahi, A.M., Ahmed, A.N., Al-khalifa, A.A. (2010). Isolation and identification of Five different date palm cultivars and the effect of Benlate fungicides in their control. Basra. J. Date Palm Res g (2). 79-97.

Altan, F., Bürün, B., & Sahin, N. (2010). Fungal contaminants observed during micropropagation of *Lilium candidum* L. and the effect of chemotherapeutic substances applied after sterilization. African Journal of Biotechnology, 9(7), 991-995.

Amiri, S., Ashtari, S., Abdullah, H. B., Nazari, S. A., Khodadadi, E., Khodadadi, E., & Sabzi, M. (2013). Control contamination during micropropagation process of rootstock Mariana (*Prunus mariana*). Annals of Biological Research, 4(3), 149-151.

Bhat, M.A., Mishra, A.K., Jan, S., Bhat, M.A., Kamal, M.A., Rahman, S., Shah, A.A., Jan, A.T. (2023). Plant Growth Promoting Rhizobacteria in Plant Health: A Perspective Study of the Underground Interaction. Plants 12, 629. https://doi.org/10.3390/plants12030629.

Bonga, J.M. (1982). Tissue Culture Techniques. In: Bonga, J.M., Durzan, D.J. (eds) Tissue Culture in Forestry. Forestry Sciences, vol 5. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-3538-4_2.

Brondani, G. E., de Oliveira, L. S., Bergonci, T., Brondani, A. E., França, F. A. M., da Silva, A. L. L., Goncalves, A. N. (2013). Chemical sterilization of culture medium: a low cost alternative to in vitro establishment of plants. Scientia Forestalis, 41(98), 257-264.

Bruna, T, Maldonado-Bravo, F, Jara, P, Caro, N. (2021). Silver Nanoparticles and Their Antibacterial Applications. Int J Mol Sci. 22(13): 7202. https://doi.org/10.3390/ijms22137202.

Chastagner, G.A., van Tuyl, J.M., Verbeek, M., Miller, W.B., Westerdahl, B.B. (2018). Diseases of Lily. In: McGovern, R., Elmer, W. (eds) Handbook of Florists' Crops Diseases. Handbook of Plant Disease Management. Springer, Cham. https://doi.org/10.1007/978-3-319-39670-5_45.

Darwesh, O. M. Eida, M. F. Matter, I. A. (2021a). Environmental Nanobiotechnology: Microbial-Mediated Nanoparticles for Sustainable Environment. Microbial Nanobiotechnology, Materials Horizons: From Nature to Nanomaterials, Springer Nature Singapore Pte Ltd. ISBN 978-981-33-4777-9, 145-164, https://doi.org/10.1007/978-981-33-4777-9_5.

Darwesh, O. M.; Ali, S. S.; Matter, I. A.; Elsamahy, T. (2021b). Nanotextiles waste management: controlling of release and remediation of wastes. Nanosensors and Nanodevices for Smart Multifunctional Textiles, ISBN: 978-0-12-820777-2, 267-286. https://doi.org/10.1016/B978-0-12-820777-2.00010-8.

Darwesh, O. M.; Eida, M. F.; Matter, I. A. (2018). Isolation, screening and optimization of L-asparaginase producing bacterial strains inhabiting agricultural soils. Bioscience Research, 15(3): 2802-2812.

Darwesh, O. M.; Matter, I. A. (2021). Nanomaterials in the biological treatment of contaminated soil. Nanomaterials for Soil Remediation, ISBN: 978-0-12-822891-3, 285- 300. https://doi.org/10.1016/B978-0-12-822891-3.00014-1.

Darwesh, O.M., Al-Balakocy, N.G., Ghanem, A. Matter, I.A. (2023a). Application of microalgal-ZnO-NPs for reusing polyester/cotton blended fabric wastes after modification by cellulases enzymes. Waste Dispos. Sustain. Energy 5(4): 471–482. https://doi.org/10.1007/s42768-023-00170-2.

Darwesh, O.M., Elshahawy, I.E. (2023). Management of sunflower charcoal-rot and maize late-wilt diseases using the aqueous extract of vermicompost (vermitea) and environmental-safe biochar derivative (wood vinegar). Sci Rep., 13: 17387. https://doi.org/10.1038/s41598-023-43974-2.

Darwesh, O.M., Eweys, A.S., Zhao, YS., Matter, I.A. (2023b). Application of environmental-safe fermentation with *Saccharomyces cerevisiae* for increasing the cinnamon biological activities. *Bioresour. Bioprocess.* 10, 12. https://doi.org/10.1186/s40643-023-00632-9.

Darwesh, O.M., Hassan, S.A.M., Abdallatif, A.M. (2023c). Improve *In vitro* Multiplication of Olive Shoots Using Environmental-Safe Chitosan, Selenium, and Silver Nanostructures. Biointerface Research in Applied Chemistry, 13(5), 419. https://doi.org/10.33263/BRIAC135.419.

Darwesh, O.M.; El-Hawary, A.S.; El Kelany, U.S.; El-Sherbiny, G.M. (2019). Nematicidal Activity of Thermostable Alkaline Protease Produced by *Saccharomonospora viridis* strain Hw G550, Biotechnology Reports, 24: e00386, https://doi.org/10.1016/j.btre.2019.e00386.

Dutra, L. F., Wendling, I., Brondani, G. E. A (2009). Micropropagação de eucalipto. Pesquisa Florestal Brasileira, Colombo, n. 58, p. 49-59.

Ebile, P.A., Opata, J., Hegele, S. (2022). Evaluating suitable low-cost agar substitutes, clarity, stability, and toxicity for resource-poor countries' tissue culture media. In Vitro Cell. Dev. Biol.-Plant, 58: 989–1001 https://doi.org/10.1007/s11627-022-10285-6.

El-Hammady, A., Wanas, W. H., El-Hamid, A. A., El-Salem, M. J. (2005). *In vitro* propagation of three almond cultivars and the almond-peach hybrid rootstock" Hansen". Arab Universities Journal of Agricultural Sciences, 13(2), 481-499.

El-Shanshoury, A. R.; Darwesh, O. M.; Sabae, S. Z.; Awadallah, O. A.; Hassan, S. H. (2020). Bio-manufacturing of selenium nanoparticles by *Bacillus subtilis* isolated from Qarun Lake and evaluation their activity for water remediation. Biointerface Research in Applied Chemistry, 10(4): 5834 – 5842. https://doi.org/10.33263/BRIAC104.834842.

Eweys, A.S., Zhao, Y-S., Darwesh, O.M. (2022). Improving the antioxidant and anticancer potential of *Cinnamomum cassia* via fermentation with *Lactobacillus plantarum*. Biotechnology Reports, 36: e00768. https://doi.org/10.1016/j.btre.2022.e00768.

Gabryszewska, E. (2005). Identification and elimination of bacterial contaminants from Pelargonium tissue cultures. *J Fruit Ornam Plant Res*, *13*, 101-8.

Grzebelus E, Skop L. (2014). Effect of β-lactam antibiotics on plant regeneration in carrot protoplast cultures. In Vitro Cell Dev Biol Plant. 50(5): 568-575. http://dx.doi.org/10.1007/s11627-014-9626-0.

Gu M, Li Y, Jiang H, Zhang S, Que Q, Chen X, Zhou W. (2022). Efficient In Vitro Sterilization and Propagation from Stem Segment Explants of *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst, a Multipurpose Woody Plant. Plants (Basel). 11(15): 1937. http://dx.doi.org/10.3390/plants11151937.

Hamed, M.A., Abass M.H. (2006). Study of cytological changes associated with contamination date palm phoenix dactylifera; Tissue cultures with fungi. Basra Res, J, 32, 1- 27.

Hasnain A, Naqvi SAH, Ayesha SI, Khalid F, Ellahi M, Iqbal S, Hassan MZ, Abbas A, Adamski R, Markowska D, Baazeem A, Mustafa G, Moustafa M, Hasan ME, Abdelhamid MMA. (2023). Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. Front Plant Sci., 14: 1197747. https://doi.org/10.3389/fpls.2023.1197747.

Hassan, S.A.M., Zaied, N.S., Hassan, H.S.A., Ahmed, D.M.M. (2019). Effective protocol for *in vitro* shoot production through flower bud explants of Jojoba (*Simmondsia Chinensis*). Plant Archives 19(1), 2631-2636.

Hegazy, R., El-Swaify, Z., & Radwan, A. (2024a). Unlocking Soil Remediation Potential: Effects of Chelators on Moringa oleifera Lam. Growth and Soil Health in Heavy Metal Contamination. *Egyptian Journal of Agronomy*.

Hegazy, R., El-Swaify, Z., & Radwan, A. (2024b). Phyto-extraction Potential of Duranta erecta's Against Heavy Metal Assisting by EDTA and Citric Acid for Mitigating Industrial Polluted Soil. *Egyptian Journal of Agronomy*.

Hesami M, Baiton A, Alizadeh M, Pepe, M, Torkamaneh, D, Jones, AMP. (2021). Advances and Perspectives in Tissue Culture and Genetic Engineering of Cannabis. Int J Mol Sci. 22(11): 5671. https://doi.org/10.3390/ijms22115671.

Hesami, M., Daneshvar, M.H., Lotfi-Jalalabadi, A. (2017). Effect of sodium hypochlorite on control of *in vitro* contamination and seed germination of *Ficus religiosa.* Iranian Journal of Plant Physiology, 7(4).

Hussain A, Ahmed I, Nazir H, et al. (2012) Plant Tissue Culture: Current Status and Opportunities. Recent Advances in Plant in vitro Culture. InTech. Available at: http://dx.doi.org/10.5772/50568.

Ijaz, I., Gilani, E., Nazir, A. and Bukhari, A., (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green chemistry letters and reviews, 13(3): 223-245.

Izarra ML, Panta AL, Maza CR, Zea BC, Cruzado J, Gutarra LR, Rivera CR, Ellis D, Kreuze JF. (2020). Identification and Control of Latent Bacteria in *in vitro* Cultures of Sweetpotato [*Ipomoea batatas* (L.) Lam]. Front Plant Sci. 11: 903. https://doi.org/10.3389/fpls.2020.00903.

Khalid, K.A., Darwesh, O.M., Ahmed, A.M.A. (2021). Peel Essential Oils of *Citrus* Types and Their Antimicrobial Activities in Response to Various Growth Locations. Journal of Essential Oil Bearing

Plants, 24(3): 480-499, https://doi.org/10.1080/0972060X.2021.1941278.

Lateef, A., Darwesh, O. M., Matter, I. A. (2021). Microbial Nanobiotechnology: The Melting Pot of Microbiology, Microbial Technology and Nanotechnology. Microbial Nanobiotechnology, Materials Horizons: From Nature to Nanomaterials, Springer Nature Singapore Pte Ltd. ISBN 978-981-33-4777-9, 1-19. https://doi.org/10.1007/978-981-33-4777-9_1.

Matter, I. A.; Darwesh, O. M.; Matter, H.A.B. (2021). Nanosensors for herbicides monitoring in soil. Nanomaterials for Soil Remediation, ISBN: 978-0-12-822891-3, 221-237. https://doi.org/10.1016/B978-0-12-822891-3.00011-6.

Mihaljević, I., Dugalić, K., Tomaš, V., Viljevac, M., Pranjić, A., Čmelik, Z., ... & Jurković, Z. (2013). In vitro sterilization procedures for micropropagation of 'OBLAČINSKA'sour cherry. Journal of Agricultural Sciences, 58(2), 117-126.

Mittal D, Kaur G, Singh P, Yadav K and Ali SA (2020) Nanoparticle-Based Sustainable Agriculture and Food Science: Recent Advances and Future Outlook. *Front. Nanotechnol.* 2:579954. https://doi.org/10.3389/fnano.2020.579954.

Mourad, R., Helaly, F., Darwesh, O.M., Sawy, S.E. (2019). Antimicrobial and physicomechanical natures of silver nanoparticles incorporated into silicone-hydrogel films. Contact Lens and Anterior Eye, 42: 325–333. https://doi.org/10.1016/j.clae.2019.02.007.

Mourad, R.M., Darwesh, O.M., Abdel-Hakim, A. (2020). Enhancing physico-mechanical and antibacterial properties of natural rubber using synthesized Ag-SiO_2 nanoparticles. International Journal of Biological Macromolecules, 164: 3243–3249, https://doi.org/10.1016/j.ijbiomac.2020.08.063.

Nair, G.M., Sajini, T. and Mathew, B., (2022). Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications. Talanta Open, 5: 100080.

Niedz, R.P., Bausher, M.G., (2002). Control of *in vitro* contamination of explants from greenhouse-and field-grown trees. In Vitro Cell. Dev. Biol. 38:468-471.

Omamor, I.B., Asemota, A.O., Eke, C.R., Ezia, E.I. (2007). Fungal contaminants of the oil palm tissue culture in Nigerian Institute for Oil Palm Research (NIFOR). African Journal of Agricultural Research, 2(10): 534-537.

Onwubiko, N.C., Nkogho, C.S., Anyanwu, C.P., Onyeishi, G.C. (2013). Effect of different concentration of sterilant and exposure time on sweet potato (*Ipomoea batatas* Lam) explants. International Journal of Current Microbiology and Applied Sciences, 2(8): 14-20.

Osterc, G., Luthar, Z., Štampar, F. (2004). The importance of sterilization procedure for producing vigorous cherry plants (*Prunus* sp.) *in vitro*. Acta Agric. Slov. 83:45-51.

Parasharami, V., Yadav, P., Mandkulkar, S. (2014). *Ficus religiosa* L.: Callus, suspension culture and lectin activity in fruits and *in vitro* regenerated tissues. British Biotechnology Journal, 4(2): 215-227.

Part, F., Zecha, G., Causon, T., Sinner, E.K., Huber-Humer, M. (2015). Current limitations and challenges in nanowaste detection, characterisation and monitoring. Waste Manag., 43, 407-420.

Pasqual, M., & Ferreira, E. A. (2007). Micropropagation of Fig tree (*Ficus carica* sp). In Protocols for micropropagation of woody trees and fruits (pp. 409-416). Springer, Dordrecht.

Pathak A, Haq S, Meena N, Dwivedi P, Kothari SL, Kachhwaha S. (2023). Multifaceted Role of Nanomaterials in Modulating In Vitro Seed Germination, Plant Morphogenesis, Metabolism and Genetic Engineering. Plants (Basel). 12(17): 3126. https://doi.org/10.3390/plants12173126.

Permadi, Nandang, Mohamad Nurzaman, Arshad Naji Alhasnawi, Febri Doni, and Euis Julaeha. (2023). Managing Lethal Browning and Microbial Contamination in *Musa* spp. Tissue Culture: Synthesis and Perspectives. Horticulturae, 9(4): 453. https://doi.org/10.3390/horticulturae9040453.

Punia, A., Singh, R.P., Singh, V. and Chauhan, N.S., (2023). Environment sustainability with microbial nanotechnology. In Environmental Applications of Microbial Nanotechnology (pp. 289-314). Elsevier.

Saleh, M. A., Zaied, N. S., Maksoud, M. A., & Hafez, O. M. (2019). Application of Arabic Gum and Essential Oils as the Postharvest Treatments of Le Conte Pear Fruits during Cold Storage. Asian Journal of Agricultural and Horticultural Research, 1-11.

Singh, S. K., Singh, A., Singh, N. V., Ramajayam, D. (2010). Pomegranate tissue culture and biotechnology. Fruit Veg. Cereal Sci. Biotechnol, 4(2), 120-125.

Singh, V., Tyagi, A., Chauhan, P.K., Kumari P., Kaushal. S. (2011). Identification and prevention of bacterial contamination on explant used in plant tissue culture labs. International Journal of Pharmacy and Pharmaceutical Sciences, 3(4): 160-163.

Taha, R.A., Hassan, S.A.M. (2016). Studies on silver nitrate impact on jojoba *in vitro* culture. International Journal of PharmTech Research., 9(8), 77-83.

Teixeira, S. L.; Ribeiro, J. M.; Teixeira, M. T. (2006). Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv *Smooth cayenne*) behavior. Plant Cell, Tissue and Organ Culture, Dordrecht, v. 86, n. 3, p. 375-378.

Telci, C., M. Yildiz, S. Pelit, B. Onol, E.G. Erkilic and H. Kendir. (2011). The effect of surface disinfection process on dormancy-breaking, seed germination, and seedling growth of *Lathyrus chrysanthus* Boiss. under *in vitro* conditions. Propagation of Ornamental Plants, 11: 10-16.

Verhaegen M, Bergot T, Liebana E, Stancanelli G, Streissl F, Mingeot-Leclercq M-P, Mahillon J and Bragard C (2023) On the use of antibiotics to control plant pathogenic bacteria: a genetic and genomic perspective. *Front. Microbiol*. 14:1221478. https://doi.org/10.3389/fmicb.2023.1221478.

Weiskirchen S, Schröder SK, Buhl EM, Weiskirchen R. (2023). A Beginner's Guide to Cell Culture: Practical Advice for Preventing Needless Problems. Cells. 12(5): 682. https://doi.org/10.3390/cells12050682.

Wolella, E. K. (2017). Surface sterilization and in vitro propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants. Journal of Biotech Research, 8, 18.

Yadav, N., Garg, V.K., Chhillar, A.K., Rana, J.S. (2023). Recent advances in nanotechnology for the improvement of conventional agricultural systems: A review. Plant Nano Biology, 4: 100032. https://doi.org/10.1016/j.plana.2023.100032.

Yılmaz, G.E.; Göktürk, I.; Ovezova, M.; Yılmaz, F.; Kılıç, S.; Denizli, A. (2023). Antimicrobial Nanomaterials: A Review. Hygiene 3: 269-290. https://doi.org/10.3390/hygiene3030020.