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The effect of diverse silver molecules and ancymidol on the number of branches and buds, and the foliage quality of the Barhee date palm, cultured *in vitro*

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Abstract
The study occurred in the Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering at the University of Basrah's College of Agriculture. Healthy, disease-free, three-year-old Barhee date palm offshoots are harvested from mature trees. The study's goal is to determine how silver nanoparticles and Ancymidol impact date palm callus growth and bud regeneration during micropropagation. Samples are gathered from the tips of secondary shoots of Barhee date palms. Modifications were made to the Murashige and Skoog (MS) medium. Auxin, cytokinin, and activated charcoal were used, as were Ancymidol (at concentrations of (0, 1, 2, and 3 mg/L^{-1}) and silver nanoparticles (at concentrations of (0, 0.150, and 0.300 mg/L^{-1}), as well as a combination of the two. The combination (3 mg/L^{-1}Ancymidol +0.300 mg/L^{-1} silver nanoparticles). Fresh and dry weight of the callus increased notably to 2.280 and 0.195 g. The embryo count was significantly influenced by both Ancymidol and silver nanoparticles, with silver nanoparticles showing a greater effect. The highest count of embryos, 14.87, was achieved with a 0.300 mg/L^{-1}dose, with Ancymidol at 3 mg/L^{-1} yielding the next highest at 12.76 embryos. Embryo levels increased significantly, reaching 15.45, when 3 mg/L^{-1} Ancymidol and 0.300 mg/L^{-1}silver nanoparticles were combined. In the control group, roots were both the least numerous and shortest, at 1 cm and 6.07 cm, respectively. The application of 3 mg/L^{-1} Ancymidol combined with 0.300 mg/L^{-1} silver nanoparticles significantly boosted root length by 2.33 cm, root number by 12.97, and leaf width by 4 leaves. The study concluded that the best approach involved using 0.300 mg/L^{-1} of silver nanoparticles and 3 mg/L^{-1} of Ancymidol in the culture medium. This combination led to improved callus formation and differentiation, as well as a higher number of embryos.

Keywords: Silver nanoparticles, ancymidol, dactylifera, tissue micropropagation efficiency

Introduction
Date palm belongs to the Arecaceae family and the Arecales order, making it a significant monocotyledonous tree. Hence, it's widespread in subtropical regions between latitudes 10 and 30 degrees north and 20 degrees south of the equator (Al-Jubouri, 2002) [2]. Tissue culture technology has been used to propagate date palms, producing numerous offshoots that are genetically identical to the mother palm. These offshoots are characterized by their vigorous growth and ease of handling. However, the most significant obstacle they face is acclimatizing the seedlings in the field (Jain, 2012) [10]. This technology provides a rapid method for plant propagation, enabling the production of large numbers of disease-free plants within a short period, free of genetic variation and identical to the parent plant. The resulting plants are small, facilitating their transport and handling (Bhattacharjee, 2006; Nimavat and Parikh, 2024) [15, 23]. Among the plant's vital processes affected by environmental stress during acclimatization is amino acid synthesis into proteins (Mengel and Kirkby, 2012) [13], which is highly efficient in the propagation's success process, increasing the germination rate of embryos and promoting seedling growth (Mazri *et al*., 2018) [12]. AgNPs are used as nanoantibiotics to stop the growth of bacteria, fungi, and viruses, according to Ibraheem *et al*. (2022) [9]. Silver nanoparticle demand has soared, leading to a limited and reasonably priced supply. As a result, the synthesis of silver nanoparticles is now seen as a major problem in nanoscience. Plant growth has been

Promoted by silver nanoparticles (Jasim *et al*., 2017) [11], which are also employed in plant protection products. Silver Nanoparticles vary in how effectively they eliminate microbes while preserving plant growth and development (Worall *et al*., 2018). Compounds that block gibberellin production are termed growth inhibitors or gibberellin biosynthesis inhibitors. Research has found that biochemical growth inhibitors are good tools for understanding the basic and additional effects within the plant, as some of these inhibitors contain a heterocyclic nitrogen ring, such as Ancymidol, fluprimidol, and paclobutrazol. Ancymidol (Ancy [α-Cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidinemethanol] is a pyrimidine analog. It inhibits the monooxygenase enzyme, which catalyzes the oxidation of ent-kaurene oxidase, a key step in the pathway of ent-kaurene in gibberellins (Rademacher 2016) [24]. Key plant processes, including bud growth and proliferation, chlorophyll accumulation, and endogenous cytokine levels, are affected by ancymidol (Rajeev *et al*., 2018) [25]. Ancymidol may promote apical dominance by decreasing the synthesis of the natural hormone auxin and enhancing cytokines (Rajeev *et al*., 2018) [25]. Ancymidol has also been shown to alter and stimulate several growth hormones (Hofmannova *et al*., 2008) [8]. In this study, we focused on investigating the effects of both nano silver and Ancymidol on the growth characteristics of the Barhi date palms *in vitro*.

Materials and Methods

An experiment was conducted in a tissue culture laboratory at the College of Agriculture, University of Basrah. For this study, we took three-year-old Barhi palm suckers from the nursery. We got these palm suckers from trees that were in excellent health, with no signs of disease or insects. The outer leaves and fibrous tissue were removed from the branch centers to prepare them for tissue culture.

The main plant materials for this investigation were branch tips, which were cut and shaped to measure 0.7-1.0 cm wide and 1-2 cm long. An antioxidant blend (100 mg/L^{-1} ascorbic acid, 150 mg/L^{-1} citric acid) was applied to the plant parts. Plant parts underwent a sterilization process: 1 minute in 70% ethanol, 20 minutes in 5% sodium hypochlorite, and finally, three rinses with sterile distilled water.

The plant parts were cultured in a medium (Murashige and Skoog 1962) [14] containing 100 mg/L $^{-1}$ glutamine, 5 mg/L^{-1} thiamine hydrochloride, 1 mg/L^{-1} biotin, 30 g/L^{-1} sucrose, and 2 g/L^{-1} activated charcoal. The medium was solidified with 6.0 g/L^{-1} agar and growth regulators, such as naphthaleneacetic acid (NAA) at 30 mg/L^{-1} and 6-(γ,γ-dimethylallyl-aminopurine) (2iP) at 3 mg/L^{-1} (Al-Mayahi 2024) [4]. The cultures were kept in complete darkness at 27±2 °C. Until callus formation was prompted, they transitioned to identical new media every six weeks. After six months, stable callus tissue was removed, weighed, and 100 mg was grown in 340 mL glass jars with 50 mL of medium saline supplemented with growth regulators NAA at 6.0 mg/L^{-1} and 2iP at 1.5 mg/L^{-1}. To test the effects of silver nanoparticles (AgNPs) and Ancymidol on callus formation, two study factors are Ancymidol concentrations of 0, 1, 2, and 3 mg/L^{-1}, and also silver nanoparticle concentrations of 0, 0.150, and 0.300 mg/L^{-1}, which were applied to MS medium. After inoculation, callus cultures were cultured at 27 ± 2 °C. A light intensity of 2000 lux was provided for 16 hours using cool-white fluorescent tubes. Callus mass was gauged eight weeks post-experiment initiation, and every treatment was given 18 replicates (neighbors).

Effect of silver nanoparticles and Ancymidol on callus growth:

Stable callus tissue was gathered and weighed after six months. Then, 100 mg of this tissue was cultivated in 340 mL glass jars containing 50 mL of MS media. These media were enhanced with 6.0 mg/L^{-1} of NAA and 1.5 mg/L^{-1} of 2iP as growth regulators. To study the effects of silver nanoparticles and Ancymidol on callus formation, different concentrations of the two compounds were added to the growth medium and evaluated. We used a concentrations of 200 mg/L^{-1}Ancymidol + 0.125 mg/L^{-1}silver nanoparticles and 200 mg/L^{-1} Ancymidol + 0.250 mg/L^{-1}silver nanoparticles, etc. Also, a control group was part of the study, and it did not receive Ancymidol or silver nanoparticles. The callus is injected in the medium & incubated at 27±2 °C. Cold-white fluorescent bulbs provided a 16-hour light period at 2000 lux. Each treatment has eighteen replicates (neighbors). Callus mass was measured eight weeks after the experiment started.

Effect of Ancymidol and silver nanoparticles on root length and number

Measured using a digital vernia

Statistical Analyses

A CRD was implemented, featuring eight distinct treatments.Using SPSS version 20, results were adjusted for normality and analyzed for variance (ANOVA) using the F-test. Comparisons of the means were made using (LSD, $p<0.05$).

Results and Discussion

Effect of silver nanoparticles and Ancymidol on callus growth

Table 1 indicates that both Ancymidol and silver nanoparticles substantially impacted callus fresh and dry weight, with higher concentrations leading to increased weight. The treatment with 0.300 mg/L^{-1} silver nanoparticles significantly increased the fresh and dry weight of callus, reaching 1.567 and 0.119 g, respectively. The treatment with Ancymidol (3 mg/L^{-1}) was effective, as it had the highest fresh and dry weight of the primary callus, with a significant difference from all the other treatments, reaching (0.741 and 0.063) g, respectively. The control treatment led to the lowest average fresh and dry weight of the primary callus (0.905 and 0.066 g, respectively). The combination (3 mg/L^{-1} anisole + 0.300 mg/L^{-1} silver nanoparticles) resulted in significant rises in the fresh and dry weights of the callus, measured as 2.280 and 0.195 g, in turn. Based on these findings, we've determined that the levels of Ancymidol and silver nanoparticles differ in the initial growth of date palm callus cultures see figure 1. Ancymidol's terminal effect resulted in greater fresh and dry callus weight. Ancymidol might inhibit apical dominance by reducing natural auxin production, according to Rajiv *et al*. (2018) [17] study on oleander.

Ancymidol has strong inhibitory effects on plant growth. The function of this is to block gibberellin (GA) creation, resulting in dwarfing. Ancymidol was found to lower endogenous auxin (IAA) synthesis in plants, as per

Hofmannova *et al*. (2008) [8]. A further finding by Hernández *et al*. (2018) [7] was that Ancymidol impacts multiple hormones in plant tissues, consequently inhibiting plant growth. Ancymidol also increases carbohydrate levels, which is reflected in increased callus fresh weight. The results may show how silver nanoparticles + Ancymidol affect tissue function in a laboratory setting.Further, the reason for the increase in the rate of fresh and dry weight may be that Ancymidol and silver nanoparticles play an effective role in increasing cell division, so increasing them causes an increase in the division of primary callus cells, which increases the fresh and dry weight of the primary callus (Ahammed, 2023) [1]. The results in Table 1 also showed that both Ancymidol and silver nanoparticles had a significant effect on the number of embryos, as there were significant differences in the number of embryos formed from 100 mg of embryonic callus, as different Ancymidol and silver nanoparticle treatments were used. The number of embryos varied across all treatments, with the silver nanoparticle treatment at 0.300 mg/L^{-1} being one example. The number of embryos varied across all treatments, with the silver nanoparticle treatment at 3.00 mg/L^{-1} being one example gave the highest rate of a number of embryos, which reached 14.87 embryos, with a significant difference from the rest of the treatments, including the control treatment, in which the number of embryos reached (5.95) embryos, followed by the treatment of Ancymidol (3.0 mg/L^{-1}). The single-dose treatment resulted in 12.76 embryos, yet the combination (3.0 mg/L^{-1}Ancymidol + 0.300 mg/L^{-1} silver nanoparticles) resulted in a significant increase to 15.45. This could be tied to the fact that using Ancymidol and silver nanoparticles in embryonic callus propagation assisted the transformation of a larger number of embryos from the spherical to the cylindrical stage (Ochatt *et al*. 2022) [16]. Alternatively, higher doses may have promoted the growth and division of embryonic callus cells (Solangi *et al*. 2020) [19]. In addition, these drugs might have fostered excellent cell growth and more date palm embryos.

The effect of Ancymidol and silver nanoparticles on the indirect formation of organs.

Table 2 indicates how Ancymidol and silver nanoparticles affect the length and number of main roots left in seedlings of the Barhee variety after 8 weeks of germination. Silver nanoparticles (0.300 mg/L^{-1}) significantly raised the number and length of roots compared to other concentrations and the control treatment (2.17 and 10.00 cm, respectively). The treatment with Ancymidol 3 mg/L^{-1} has the most effect, as it recorded the highest rate of root number and length, with a significant difference from the sum of the other treatments, reaching 1 and 7.23 cm, respectively. The control treatment resulted in the lowest roots (1 and 6.07 cm, respectively). The combination of 3 mg/L^{-1} Ancymidol and 0.300 mg/L^{-1} silver nanoparticles significantly boosted root number and length, plus leaves number, to 2.33, 12.97 cm, and 4 leaves, respectively.

The superiority of Ancymidol and silver nanoparticle concentrations (in root length and number) added to the nutrient medium for vegetative embryo maturation and germination, as well as the characteristics of the resulting seedlings, may be attributed to the accumulation of carbohydrates at these concentrations in high quantities within plant cells Figure 2. Carbohydrates play a crucial role in osmotic regulation and serve as an energy source, positively influencing the number of cell divisions. They are also essential for protein synthesis, which is necessary for the development, growth, germination, and maturation of vegetative embryos into complete plants (Vale *et al*., 2018) [21]. Another potential role for ancymidol is to reduce apical dominance, leading to dwarfing. It may also decrease endogenous auxin (IAA) production in some tissues. (Rajeev *et al*., 2018) [25] Ancymidol has been found to alter several plant growth hormones and increase glucose levels in meristematic tissue centers and bud growth. Vaidya *et al*. (2019) [20] reported similar results, showing that adding ancymidol to the culture medium significantly increased the growth of mint buds. These findings overlap with the effects of silver nanoparticles on palm tissue culture. Silver nanoparticles can affect plant growth by managing alterations in water and nutrient levels, according to Zuverza-Mena *et al*. (2016) [22]. In their 2021 work, Hegazi *et al*. 2021 [6] observed that nano-silver enhanced shoot length, explant numbers, and the quantity of leaves and shoots in olive tissue culture.

Table 1: Influence of ancymidol and silver on callus characteristics and embryo numbers.

Treatments (mg/L^{-1})	Callus fresh weight (g)	Callus Dry weight (g)	Number of embryos
0.0 control	0.905	0.066	5.95
0.150 AgNPs	1.083	0.087	12.32
0.300 AgNPs	1.567	0.119	14.87
1.0Ancy	0.371	0.026	10.34
2.0Ancy	0.549	0.043	11.29
3.0 Ancy	0.741	0.063	12.76
2.0 Ancy +0.150 AgNPs	2.021	0.174	14.26
3.0 Ancy + 0.300AgNPs	2.280	0.195	15.45
R. LSD 0.05	0.0256	0.0072	0.930

Table 2: Influence of ancymidol and silver on the length and number of roots and leaflets

Treatments (mg/L^{-1})	Number of roots	Root length (cm)	Number of leaflets
0.0 control	1	6.07	2
0.150 AgNPs	1	8.76	3
0.300 AgNPs	2.17	10.00	3
1.0Ancy	1	6.23	2.67
2.0Ancy	1	7.06	3
3.0 Ancy	1	7.23	3
2.0 Ancy +0.150 AgNPs	2	12.40	3.67
3.0 Ancy + 0.300AgNPs	2.33	12.97	4
R. LSD *0.05*	0.416	0.577	0.833

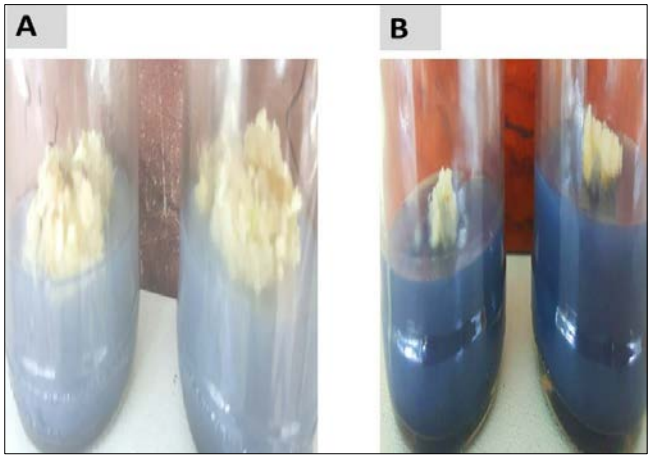

Fig 1: Callus proliferation on MS medium with. (A) 2.0 Ancy +0.150 AgNPs, (B) control treatment

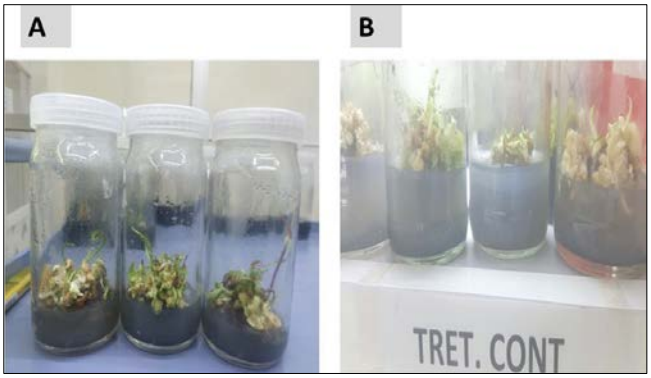

Fig 2: Number of leaflets on MS medium with. (A) 2.0 Ancy +0.150 AgNPs, (B) control treatment

Conclusions and Recommendations

This study aimed to determine the effect of Ancymidol and silver nanoparticles in the culture medium on the micropropagation efficiency of *in vitro*-grown palm suckers. According to the study, the micropropagation efficiency was improved by adding silver nanoparticles, both with and without ancymidol. The most effective result was seen when 300 mg/L silver nanoparticles +3 mg/L ancymidol were added to the culture medium. This leads to better callus development and differentiation, as well as increased micropropagation efficiency.Additionally, these levels were ideal for fostering plant development. The outcome was the greatest fresh and dry callus weights, along with the highest number of embryos. Furthermore, the addition of 300 mg/L silver nanoparticles and 3 mg/L Ancymidol to the medium increased root quantity and length, along with greater leaflet formation. We suggest, consequently, employing diverse concentrations of silver nanoparticles or Ancymidol across other mediums and tissue-cultured plant varieties to gauge their influence on growth attributes.

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