Optimizing silver nitrate concentration for enhanced Date Palm (*Phoenix dactylifera* L.) Tissue culture propagation and contamination management: A quantitative analysis

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Abstract:

Date palm (*Phoenix dactylifera* **L.***)* is a crucial crop in arid and semi-arid regions globally, prized for its fruit and resilience. However, in vitro propagation is often hindered by microbial contamination and suboptimal growth conditions. Silver nitrate ($AgNO_3$), renowned for its antimicrobial properties, has been employed as an effective agent in the tissue culture. This study investigates the optimal $AgNO_3$ concentration required for achieving a balance between contamination control and plant growth promotion in the date palm tissue culture. By analyzing a range of $AgNO_3$ concentration (0-625µg/L), we found that 250 µg/L $AgNO_3$ yielded the highest performance reducing contamination to 13% promoting a shoot elongation to 5.6cm and achieving 85% root induction. Higher concentration (≥ 375µg/L) resulted in phytotoxicity and inhibited growth. The findings suggest that 250µg/L is the optimal concertation for maximizing tissue culture success and minimizing contamination in the date palm propagation.

Keywords: Silver Nitrate, Contamination Management Phytotoxicity, Date Palm Tissue Culture, *Phoenix Dactylifera.*

Introduction: Date palm hold the immense agricultural and economic significances, particularly in the arid regions. Its successful mass propagation relies on the in-vitro techniques, which are often compromised by the microbial contamination and variability in the growth responses.(1)(2)

Silver nitrate is known for its dual role in the mitigating microbial activity and modifying ethylene-mediated responses, making it a promising candidate for improving tissue culture outcomes.(3)(4)

This study aims to explore the effects of $AgNO_3$ in controlling contamination and promoting the developmental parameters in the date palm cultures. By systematically varying its concentration in the culture medium, we sought to establish its optimal dosage for maximal efficacy. From this it will establish the understanding of the concentration-dependent effects of $AgNO_3$ will help refine tissue culture protocols and promote more efficient and reproducible results.

Material and Methods

1. **Plant material**: Embryos and explants from off shoots of the date palm (Elite Kutch) cultivated in Bhuj, India. The samples underwent surface sterilization using a sequential treatment of 70% ethanol for 2 minutes, followed by 0.1% mercuric chloride for 10 minutes. After sterilization. Explants were thoroughly rinsed with sterile distilled water to remove any residual disinfectant. The explants were cultured on MS Medium supplemented with various concentration of $AgNO_3$ (0,125,250,375,625 µg/L). The medium was supplemented with 6-benzylaminopuine (BAP) as a growth regulator for shoot induction. Cultures were maintained in a growth chamber at 25°C ± 2°C under a 16-hour light/8-hour dark photoperiod.

2. **Experimental design**:
   A *completely randomized design* (CRD) was adopted, with five treatments and three replications per treatment (n=3).(5). Each treatment contained 15 explants. The cultures were monitored for microbial contamination, shoot elongation, and root induction at 4,8, and 12 weeks. $AgNO_3$ was introduced into the culture medium at five concentrations: 0, 125, 250, 375, 500, and 625 µg/L. Two parameters were evaluated:
   1. **Contamination-free cultures:** It was assessed by visual inspection of microbial growth, recorded as a percentage of contaminated cultures at 72 hours post culture initiation.
   2. **Shoot elongation:** The length of the longest shoot was measured at 4 and 8 weeks to assess shoot growth.
   3. **Root Induction:** The percentage of explants with developed roots was recorded at 8 and 12 weeks.
      The effectiveness index was calculated to determine the optimal $AgNO_3$ concentration using the formula. (6)

$$\text{Effective index} = \frac{\text{Success rate} \times \text{Root induction rate}}{100 - \text{contamination rate}}$$

Statistical Analysis:

Data were analyzed using one-way ANOVA and t-test with the control group (0 ug/L) to identify significant differences between treatments. Post Hoc comparisons were conducted using the Tukey HSD test ($p<0.05$). All statistical analyses were performed using IBM SPSS 21.0 software

Results and Discussion

Microbial Contamination control: At 250µg/L $AgNO_3$ the concentration rate was reduced to 13%, which was significantly lower than the control group with 55% contamination**.** Higher $AgNO_3$ concentration (375µg/L and 625µg/L) resulted in increased contamination rates, likely due to phytotoxicity at these levels, which adversely affected explant health and compromised contamination control.(7)(8)

Shoot Elongation and root induction: Explants cultured with 250µg/L AgNO3 exhibited the highest shoot elongation (5.6cm) and 85% root induction. This concentration provided the most favorable conditions, promoting both shoot and root development without causing phytotoxicity. In contrast, higher $AgNO_3$ concentration (375 µg/L and above) resulted in significant growth inhibition, with reduced shoot elongation and root induction. The growth inhibitory effect of $AgNO_3$ at these concentrations was evident by the stunted shoots and poor root development with root induction rates falling below 30% at 625 µg /ml.(9)

Effectiveness index: The effectiveness index was highest at 250µg/L $AgNO_3$ with a value of 4.87, indicating the optimal balance between contamination control and growth promotion. Concentrations above 375 µg/L shown a reduction in the effectiveness index, highlighting the detrimental effects of higher $AgNO_3$ concentration on plant growth. (10)

Underlying Mechanism: The antimicrobial action of silver nitrate stems from its ability to disrupt the microbial cell ways and interfere with the DNA replication mechanism. Besides, its role in the modulating the ethylene responses may explain the observed enhancements in the plant morphogenesis, as the ethylene often inhibits the growth under in vitro conditions.(11)

This study highlights the concentration-dependent effects of silver nitrate ($AgNO_3$) on date palm tissue culture, demonstrating that 250µg/L $AgNO_3$ provides the optimal concentration for controlling the microbial contamination while simultaneously promoting growth. The antimicrobial properties of $AgNO_3$, are critical in maintaining sterile conditions necessary for successful tissue culture propagation.(12)

At concentration below 250µg/L. $AgNO_3$ showed insufficient antimicrobial efficacy allowing contamination to persist. Conversely concentration above 375µg/L resulted in phytotoxicity, which manifested as stunted growth and inhibited root development consistent with the findings in other plant species (**Table 1**).

The mechanism of $AgNO_3$'s action appears to involve its ability to inhibit the microbial growth while maintain plant cell viability. This study also suggests that ethylene inhibition plays a significant role in promoting growth under $AgNO_3$ treatment. (13)

Ethylene, a plant hormone associated with the stress is known to inhibit the root and shoot development. By reducing ethylene action, $AgNO_3$ helps in optimal growth and regeneration.(14) (15) (Table 2)

Table 1

Effect of different Concentration of $AgNO_3$ in the culture media			
	Contamination percentage		
$AgNO_3$ concentrations (µg/L)	**Establishment Stage**	**Callus stage**	**Multiplication & germination stage**
0	20.11	15.44	11.15
125	15.44	11.15	10.31
250	10.32	10.31	7.21
375	7.21	7.21	4.32
500	4.35	4.32	2.24
625	2.26	2.24	0.00

$$Callus\ induction\ \% = \frac{number\ of\ explant}{total\ number\ of\ explants} \times 100$$

Table 2

Effect of Silver Nitrate on callus stage			
$AgNO_3$ CONCENTRATIONS (µg/L)	**CALLUS FORMATION %**	**CALLUS GROWTH**	**GLOBULAR EMBRYO FORMATION**
0	40.88	1.77	8.00
125	45.55	2.66	17.00
250	58.77	3.11	14.00
375	62.66	3.44	12.00
500	70.33	3.88	7.00
625	74.44	4.22	8.00

$$Direct\ embryo\ Percentage = \frac{\text{number of explant forming direct embryo}}{\text{total number of embryo}} \times 100$$

Table 3:-

Effect of Silver Nitrate on date palm during embryo stage			
$AGNO_3$ CONCENTRATIONS (µg/L)	**Direct embryo formation %**	**Embryo multiplication**	**Embryo germination**
0	30.11	0.33	0.33
125	35.22	0.48	0.50
250	43.22	0.55	0.65
375	48.22	0.68	0.72
500	48.55	0.88	0.81
625	68.11	1.07	0.95

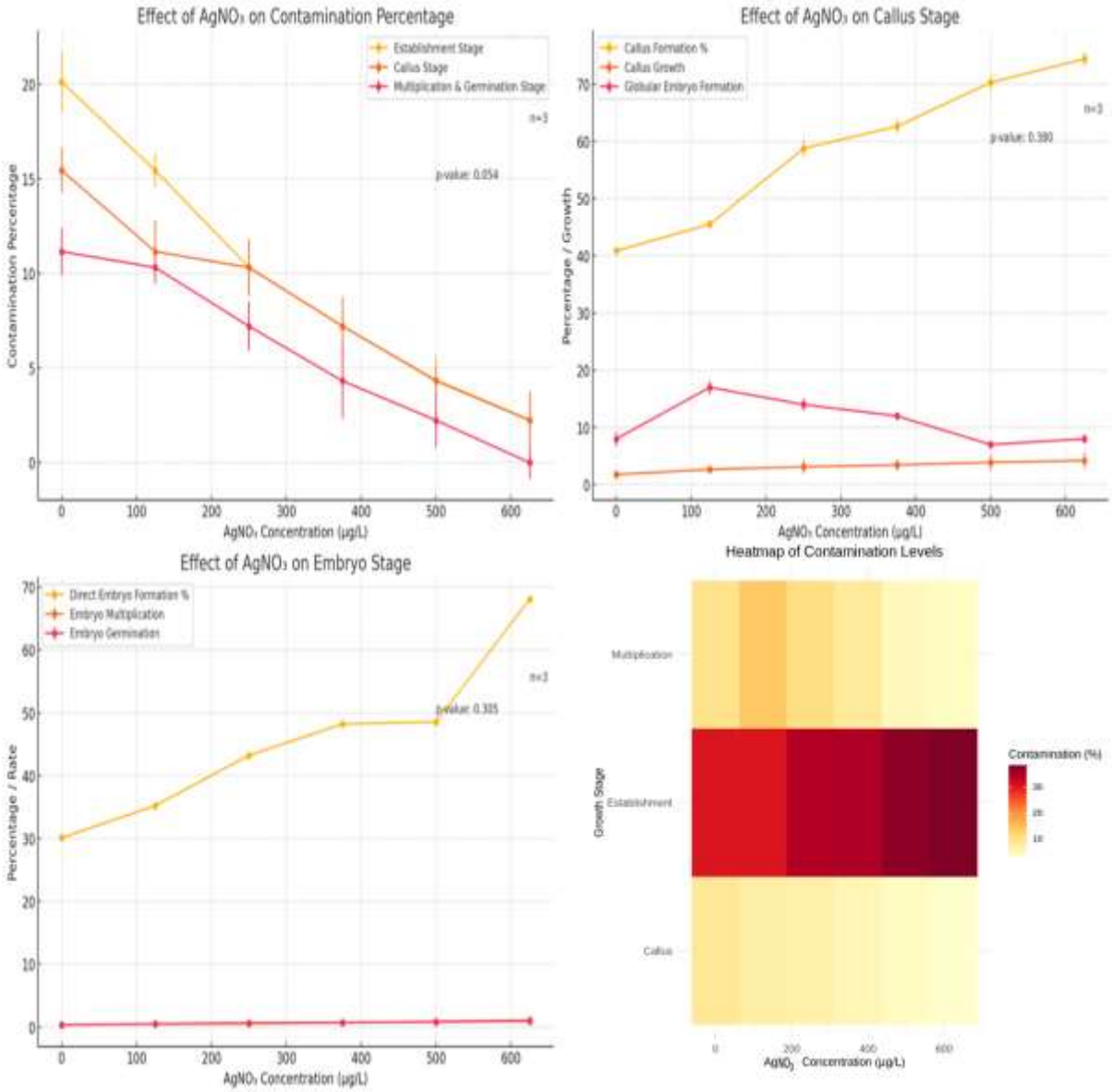

Fig.1: The contamination percentgaes at different $AgNO_3$ concentration (0-625ug/L) across the samples (n=3) tissue culture stages : establishment,callus,and mulitplcation and germination. A siginificant reduction in the contamination was observed with the increasing concentrations,with the lowest contamiantion rates recorded at 625ug/l. Statitical analysis revealed that the differences between 250ug/l and higher concentration (375-625ug/L) was significant for contaminaitonreduction,but higher concentration showed the potential phytotoxic effects as disccused in the text. **Fig.2:** Effect of $AgNO_3$ on the globular embryo formation in the date palm tissue culture-Illustrates the globular embryo formation percentages as influenced by $AgNO_3$ concentrations. The formation peaked at 125ug/l (17%) followed by a decline with higher concentrations. Statistical analysis (ANOVA, $p<0.380$) indicate that embryo formation at 125ug.L was significantly higher than the that at 500-625 ug/L, highlighting potential inhibitory effects of elevated $AgNO_3$ levels.

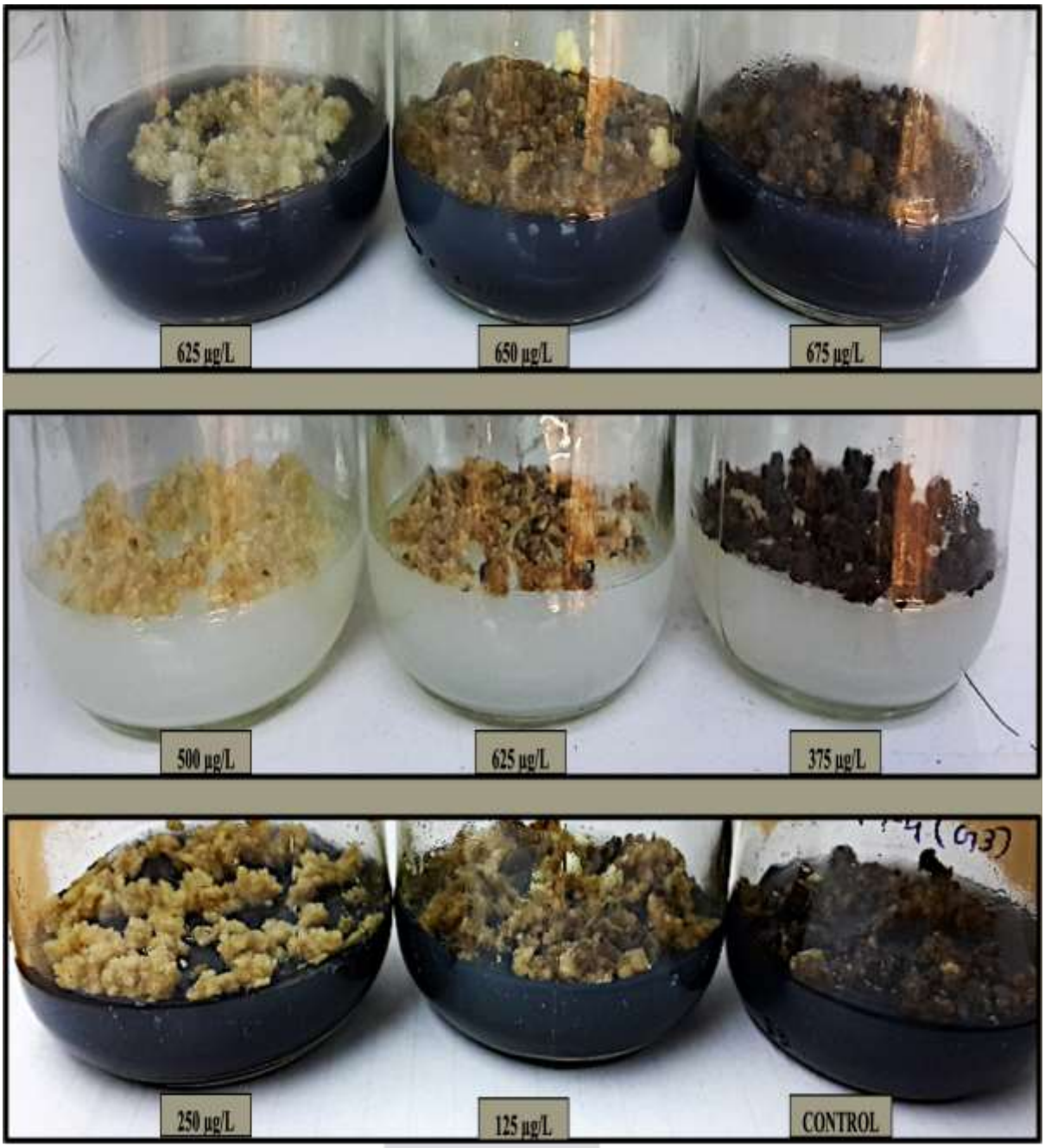

Fig. 3(A) Effect of AgNO3 on the induction ,growth and somatic embryogenesis germination of the Kutch elite date.

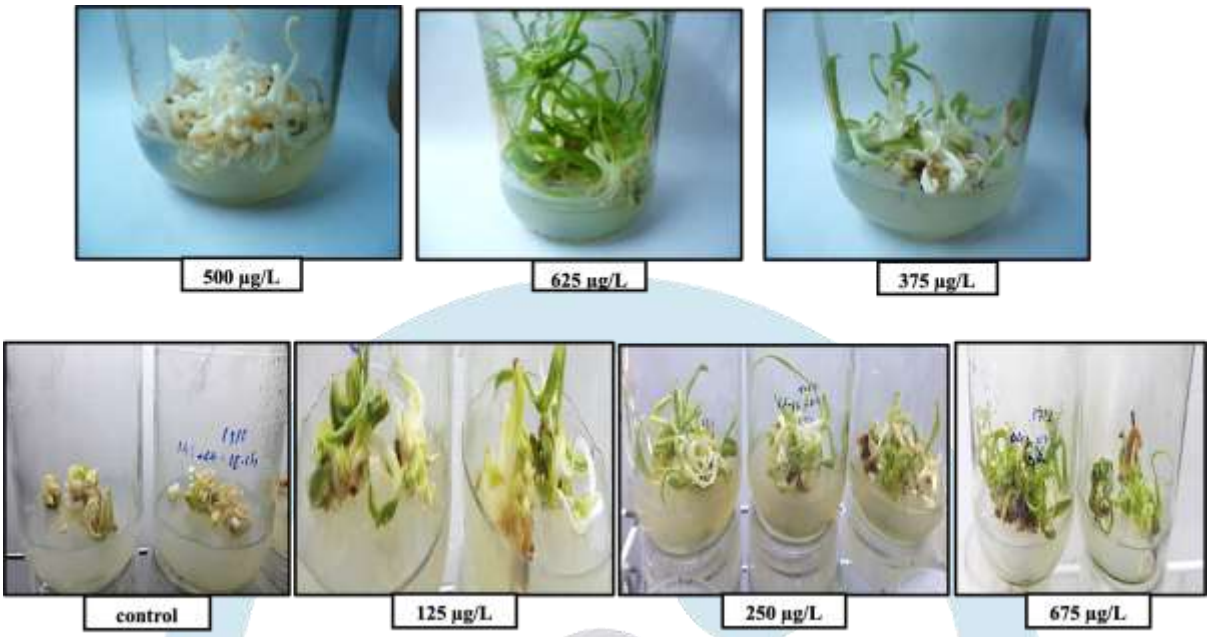

Fig .3 (B) Effect of AgNO3 on the embryo formation,mulitplication and somatic embryogenesis germination of the Kutch elite date

Conclusion: The findings of this study indicates that 250µg/L $AgNO_3$ is the optimal concentration for the contamination control and growth promotion in date palm tissue culture. At this concentration microbial contamination was minimized, while shoot elongation and root induction were maximized. The results demonstrate that increasing the concentrations of AgNO3 reduce the contamination rates and enhance the callus formation, growth and embryo development. The most significant improvements were observed at 625ug/L, with contamination percentage dropping to a significant lower stage. However, higher concentrations also led to a reduction in the globular embryo formation, indicating a potential inhibitory effect beyond a certain threshold

The results provide important insights for optimizing in vitro propagation protocols for the date palm ultimately improving the efficiency of propagation and enhancing the quality of plantlets produced.

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