Effect of the plant preservative mixture (PPM) and Ginger Extract on contamination prevention and Vegetative growth in date palm. (*Phoenix dactyliferous*)

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Abstract:

The propagation of date palm (phoenix dactylifera) through micropropagation is an essential strategy for producing the high-quality uniform plants. However, the bacterial contamination is a persistent challenges in tissue culture systems, negatively impacting efficiency and plantlet survival. This study explores the efficacy of plant preservative mixture (PPM) and ginger (Zingiber officinale) extract as antimicrobial agents to control contamination and enhance vegetative growth during date palm micropropagation. Treatments concentrations ranging from 0 to 9ml/L were applied, with outcomes assessed during the multiplication and rooting stages. Thes results demonstrated significant contamination control and optimal vegetative growth at 8ml/L for both the agents. These findings establish PPM and ginger extracts as effective, eco-friendly alternatives to antibiotics, providing sustainable solutions for micropropagation protocols.

Keywords: *Date Palm, Ginger, Contamination, Plant preservative mixture, Micropropagation*

Introduction:

Date palm (*Phoenix dactyliferous*) holds a substantial economic and socio-cultural importance in the arid and semi-arid regions, serving as a primary source of nutrition and livelihood. Tissue culture is widely adopted for its propagation to ensure the production of genetically uniform, disease-free plantlets. However, bacterial contamination during tissue culture propagation hinders its successful multiplication. (1)

Traditional methods to mitigate contamination rely heavily on antibiotics like tetracycline and chloramphenicol. Although effective these methods have several drawbacks, including the development of antibiotic-resistant bacteria and potential phytotoxicity. Therefore, there is an increasing need for natural, sustainable alternatives with broad-spectrum antimicrobial properties. (2)

Plant preservative mixture (PPM) and ginger extract have shown the promise as natural antimicrobial agents. PPM is a commercially available product known for its ability to inhibit microbial growth, while ginger extract contains bioactive compounds such as phenols, flavonoids, gingerols, and shogaols, which have proven antimicrobial activity. Despite their potential, their application in the date palm tissue culture remains underexplored.(3) (4)

This study aims to:

1. Evaluate the antimicrobial efficacy of PPM and ginger extract in date palm micropropagation.

2. Assess their impact on vegetative growth parameters during the multiplication and rooting stages.
3. Identify optimal concentration for contamination control and growth promotion.

By addressing these objectives, this research contributes to the development of eco-friendly micropropagation protocols for date palm and other economically important crops.

Material and Methods

Plant Material:

Explant, calluses, and shoot cultures (measuring 2-3cm in length) were collected during the multiplication stage of date palm micropropagation. Each cluster consisted of 3-4 shoots. Cultures were inspected meticulously for signs of bacterial contamination, identified as white or creamy colonies with a cloudy appearance on the culture medium surface. Contaminated or suspicious cultures were immediately (Figure 3) to ensure experimental accuracy.

Preparation of Ginger Extract:

Fresh ginger rhizomes (Zingiber officinale) were washed thoroughly, peeled and sliced into thin pieces using sterile tools. The slices were air-dried for 10-12 hours at room temperature, reducing their weight to approximately 20g.These dried slices were combined with 180ml of sterile water, bringing the total volume to 200ml.The mixture was boiled for 10-15 minutes and allowed to cool for 1 hour. Filtration was conducted using a vacuum filtration assembly, and the residue was re-extracted twice. The final filtrate were stored in sterile glass bottles at 4-6 degree for further use.(5)

Experimental Design:

Two experimental groups were established to study the effects of PPM and ginger extracts:

1. PPM Group: Media supplemented with PPM at concentration of 0,2,4,6, and 8ml/L.

2. Ginger extract group: Media supplemented with ginger extract at the same concentrations.

The multiplication medium comprised full-strength MS salts (Murashige & Skoog,1962), supplemented with 50g/L sucrose,0.5mg/L thiamine-HCL, 100mg/L myo-inositol,0.1mg/L biotin,0.5mg/L pyridoxine,0.5mg/L nicotinic acid, and 170mg/L NaH2PO4 with 0.05mg/L 2-isopentenyl adenine (2-iP) as a plant growth regulator. For rooting the medium included 3g/L activated charcoal and 1mg/L NAA. The pH of all media was adjusted to 5.7+-0.1 before the addition of 6g/L agar, and media were dispensed into jars (40ml per jar for multiplication and 350 ml for rooting). Sterilization was achieved by autoclaving at 121 dgree celcius and 15psi for 20 minutes.

Cultivation and Maintenance:

Cultures were incubated at 27 $\pm$ 2 ° C under a 16-hour photoperiod with light intensities of 1500 lux (multiplication) or 2500 lux (rooting). Subcultures were performed every 8 weeks for multiplication and every 4 weeks for rooting.

Treatments: PPM and Ginger extract were tested as concentrations ranging from 0 to 8ml/L for their effects on contamination and vegetative growth. These concentrations were selected based on the prior studies and preliminary experiments indicating their effectiveness in the similar tissue culture applications.

Parameters Measures

1. **Contamination prevention**: Metrics for explant, callus, embryo, and shoot contamination levels.
2. **Shoot vegetative growth**: Average number of shoots, shoot length, and growth vigor.
3. **Root vegetative growth**: Plantlet length, root number, and root length.

Statistical Analysis:

One-way ANOVA was performed to analyze significant differences across concentrations followed by the graphical representation. Post-hoc tests, such as Tukey's HSD, were conducted to identify specific group differences and ensure robust interpretation of the results.

Results:

Shoot Vegetative Growth:

It improved with increasing concentrations of PPM and ginger extract, peaking at 8ml/L. The average number of shoots increased from 32.44 to 41.22, while shoot length and growth vigor followed a similar trend.

Root Vegetative Growth:

It involves the plantlet length ,root number ,and root length, improved significantly at higher concentrations of PPM, with the optimal values recorded at 8ml/L.

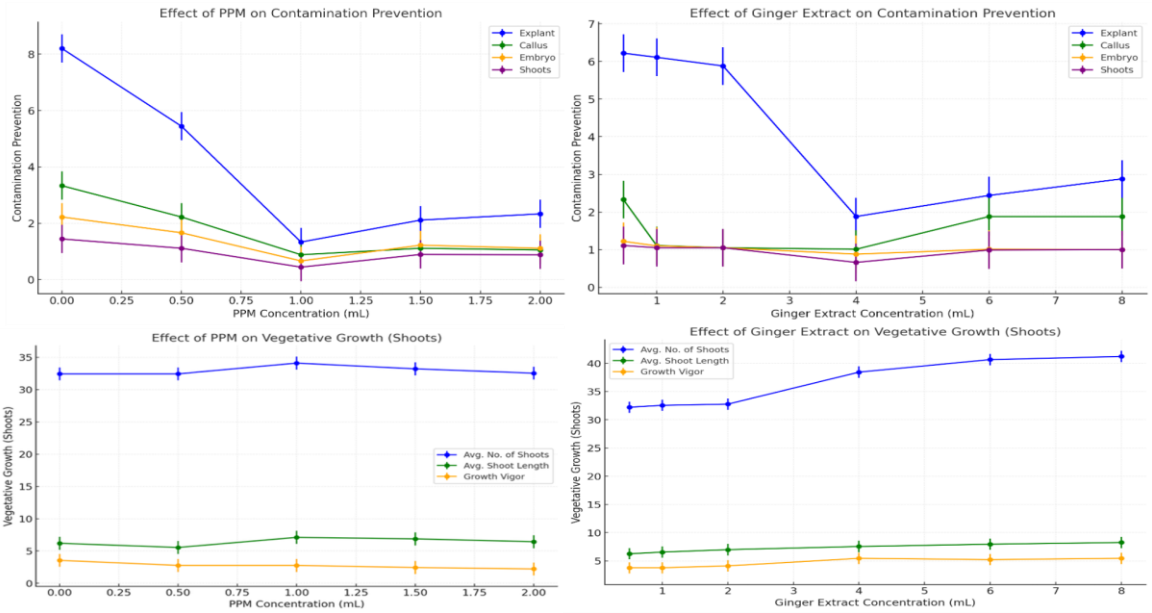

***Fig.1**: Effect of PPM and ginger extract on contamination prevention and shoot growth in plant tissue culture. (A) PPM reduced contamination in explants, callus, and embryos and shoots, with the most significant reduction observed at 0.5ml-1.0ml. (B) Ginger extract showed optimal contamination prevention at 4ml, with the explants showing the greatest response. (C) PPM had minimal impact on shoot number, length, and vigor across testes concentrations. (D) Ginger*

extract significantly increased shoot number ($p<0.001$), with the highest growth recorded at 8ml.Error bars represent standard deviation from the mean of three replicates. Statistical significance was assessed via one-way ANOVA, with $p<0.05$ considered significant.

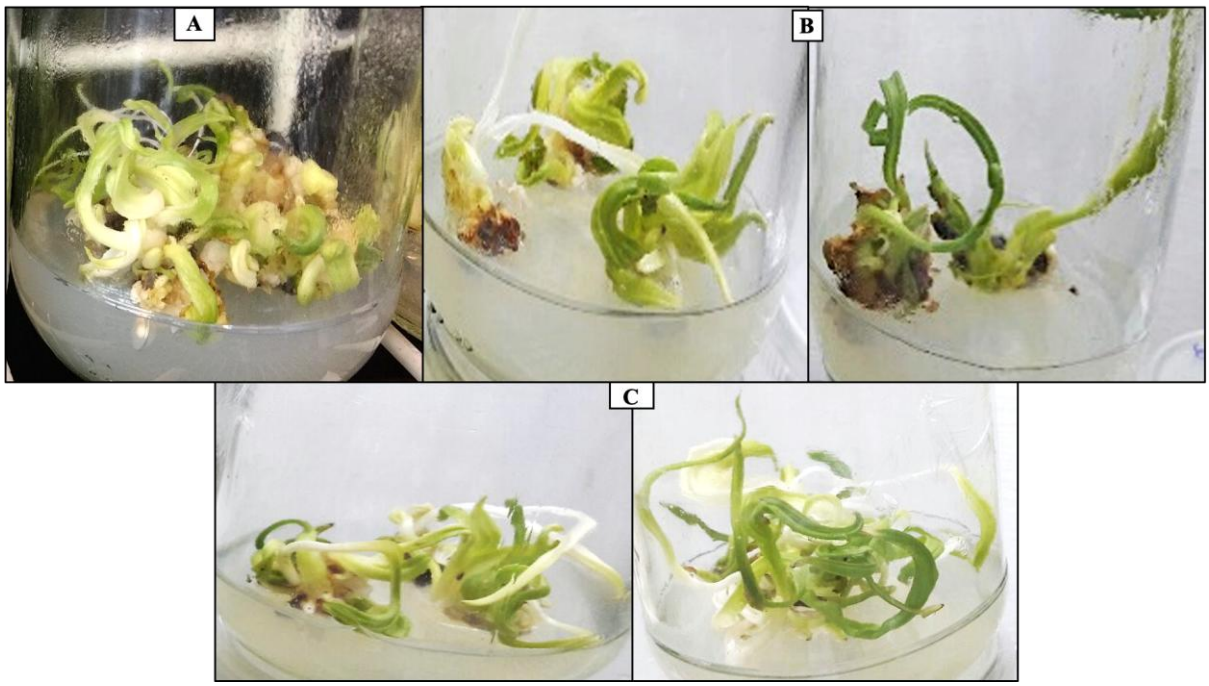

Fig 2 : *Morphological response of explants to PPM and ginger extract treatments in tissue culture.* ***(A)****Explants treated with PPM (0.5-1ml) showing healthy shoot proliferation with minimal contamination. The treatment effectively prevented browning and promoted shoot formation.* ***(B)*** *Explants cultured with the lower concentration of PPM (≤ 0.25ml) showing moderate shoot elongation but with some browning and contamination.* ***(C)*** *Explants treated with ginger extract (4-8ml) showing vigorous shot proliferation, elongated green shoots, and minimal signs of contamination. Higher concentration of ginger extract enhanced shoot growth while reducing browning. These morphological observations align with the graphical data, where PPM (0.5-1.0ml) and ginger extract (4-8ml) were the most effective in preventing contamination and promoting shoot proliferation*

Discussion: The study highlights the dual role of PPM and ginger extract in reducing the contamination and promoting the vegetative growth in date palm tissue culture. PPM's antimicrobial properties contributed to reduced the contamination levels, aligning with findings from previous research. Similarly, the presence of secondary metabolites in ginger extract, such as flavonoids and phenols, likely enhanced its antimicrobial efficacy. (6)(7). Optimal effects were observed at 8ml/L for both PPM and ginger extract treatments, indicating a concentration-dependent response. The improvements in growth metrics were gradual from lower concentration to 8ml/L, with noticeable plateau effect beyond this point. For instance, increasing ginger extract concentrations beyond 8ml/L did not yield further significant enhancements, suggesting that this concentration is optimal for achieving both contamination control and vegetative growth. These findings are consistent with previous studies on the use of plant -derived antimicrobials in tissue culture systems (8)(9)

Effect of PPM (plant preservative mixture) and Ginger extract on contamination prevention									
PPM Conc.(ml)	Explant	Callus	Embryo	Shoots	Ginger extract	Explant	Callus	Embryo	Shoots
0	8.2	3.33	2.22	1.44	0.5	6.22	2.33	1.22	1.11
0.5	5.44	2.22	1.66	1.11	1	6.11	1.11	1.1	1.05
1	1.33	0.88	0.66	0.44	2	5.88	1.05	1.05	1.05
1.5	2.11	1.11	1.22	0.89	4	1.88	1.01	0.88	0.66
2	2.33	1.05	1.11	0.88	6	2.44	1.88	1.01	0.99
NA	NA	NA	NA	NA	8	2.88	1.88	1	1

Effect of PPM & Ginger extract on vegetative growth of date palm.							
PPM conc (ml)	Avg. no. of shoots	avg. shoot length (CM)	Growth Vigor	Ginger conc. (mL)	Avg. no. of shoots	avg. shoot length (CM)	Growth Vigor
0	32.44	6.2	3.55	0.5	32.22	6.25	3.77
0.5	32.44	5.55	2.77	1	32.55	6.55	3.77
1	34.11	7.11	2.77	2	32.77	6.98	4.11
1.5	33.22	6.88	2.44	4	38.44	7.55	5.44
2	32.55	6.44	2.22	6	40.66	7.95	5.22
NA	NA	NA	NA	8	41.22	8.25	5.44

Effect of PPM & Ginger extract on vegetative growth of date palm.							
PPM CONC.(ml)	Avg. Plantlets length (cm)	avg. root no.	Avg. root length (cm)	Ginger conc. (mL)	Avg. Plantlets length (cm)	avg. root no.	Avg. root length (cm)
0	15.1	4.5	4.9	0.5	15.3	4.9	5.2
0.5	14.9	4.7	4.5	1	15.8	5.4	5.2
1	15.5	5.5	4.3	2	16.2	5.9	5.7
1.5	15.2	5.1	4	4	17.5	6.5	5.9
2	15.2	5.3	4	6	17.2	5.9	6.1
NA	NA	NA	NA	8	17.1	5.9	6.1

Fig.3*: Stages of bacterial contamination in date palm tissue culture. The image illustrates the progressions of bacterial contamination observed during tissue culture experiments. The upper image depicts the explant showing varying degrees of contamination and tissue browning. The lower images demonstrate the stages of bacterial contamination:* ***Initial stage:*** *Early colonization with the visible tissue discoloration and browning.* ***Advanced stage:*** *Full contamination, characterized by extensive tissue browning, necrosis, and the presence of bacterial growth in the culture medium. These observations highlight the importance of optimizing antimicrobial treatments to prevent contamination while promoting the healthy tissue growth.*

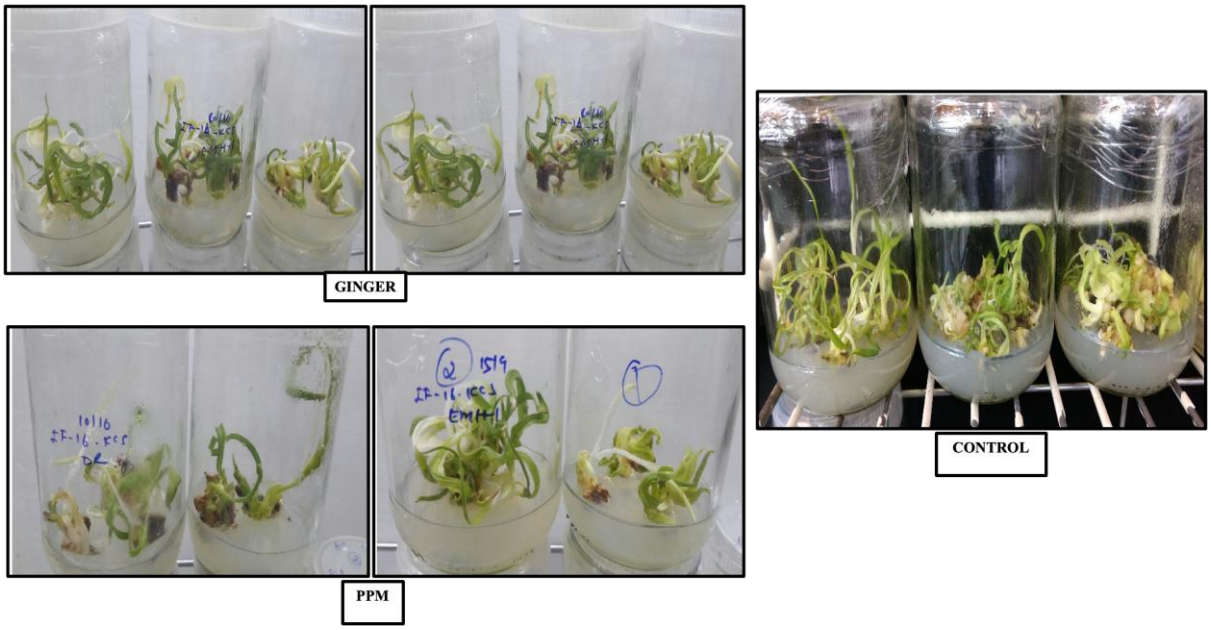

Fig 4*.: Control group: The growth of the date palm cultures in the control group was documented for the baseline comparisons.* ***Control group****: The growth of the date palm cultures in the control group was documented for baseline comparisons.* ***Ginger Extract treatment****: it as treated with ginger extract demonstrated effective contamination control.*

Furthermore, the growth of the cultures in this group was significantly better compared to PPM treatment. PPM (Plant Preservative Mixture) treatment: Cultures treated with PPM showed slower growth in comparisons to those treated with ginger extract, although contamination was also managed effectively

Conclusion

PPM and ginger extract demonstrate the robust potential as eco-friendly alternatives to conventional antibiotics in the date palm tissue culture. (10).

The optimal concentration of 8ml/L ensures minimal contamination and promotes superior vegetative growth, likely due to the balance of antimicrobial efficacy and minimal phytotoxic effects. This concentration aligns with prior studies that demonstrated similar effectiveness of moderate concentrations of plant derived antimicrobials in tissue culture systems, suppressing the results of lower concentrations or conventional antibiotics. (11)(12)

Further research should explore long-term field performance and the applicability of these treatments to other crop species, particularly those with similar tissue culture challenges such as banana (*Musa spp*), grapevine (*Vitis vinifera*) and potato (*Solanum tuberosum*)(13)(14)

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