International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 10 Issue: 11 | Nov - 2026 SJIF Rating: 8.659 ISSN: 2582-3930

Effect of Different Concentrations of Bavistin on Contamination Control and In Vitro Establishment of Banana (*Musa Acuminata* L.)

Dr. Vipul Hemant Phalak[1], Mr. Manoj Hemant Phalak[2], Prof. Kishor Sukhlal Vishwakarma[3]

[1]Department of Biochemistry, Moolji Jaitha College (Autonomous), Jalgaon, Maharashtra.
[2]Department of Biochemistry, School of Life Sciences, KBCNMU, Jalgaon, Maharashtra.
[3] Department of Biochemistry, School of Life Sciences, KBCNMU, Jalgaon, Maharashtra.

Abstract

The present study was conducted to evaluate the effect of different concentrations of Bavistin (Carbendazim) on contamination control and explant survival in banana (*Musa acuminata* L., Grand Naine/G9) using sucker explants under in vitro conditions. Five concentrations of Bavistin (1%, 2%, 3%, 4% and 5%) were evaluated using three replicates, with 10 explants per replicate. Observations were recorded for contamination, explant survival and browning or mortality. The results revealed that 1% Bavistin exhibited the highest explant survival (86.67%), although 13.33% contamination was observed. Treatment with 2% Bavistin resulted in complete contamination control with 70% explant survival. Further increases in Bavistin concentration resulted in a progressive decline in explant survival, reaching 53.33%, 43.33% and 16.67% at 3%, 4% and 5%, respectively. Correspondingly, browning and mortality increased with increasing Bavistin concentration, with the highest percentage (83.33%) recorded at 5%. Statistical analysis revealed a highly significant difference in explant survival among the treatments (one-way ANOVA, F = 79.625, p less than 0.001). The findings suggest that Bavistin concentration significantly influences contamination control and explant viability in banana sucker explants. Among the tested treatments, 2% Bavistin provided the most promising balance between contamination control and explant survival and may therefore be considered a suitable concentration for further optimization of in vitro establishment protocols for banana cv. Grand Naine (G9).

Keywords: Banana, *Musa acuminata*, Grand Naine (G9), sucker explants, Bavistin, Carbendazim, in vitro culture, contamination control, explant survival, browning.

1. INTRODUCTION

Banana (*Musa acuminata Colla*) cv. Grand Naine (G-9) is one of the commercially important dessert banana cultivars, widely cultivated because of its high yield potential, desirable fruit quality, and suitability for domestic and international markets. The increasing demand for uniform, disease-free, and high-quality planting material has encouraged the adoption of in vitro micropropagation techniques for large-scale banana production. Plant tissue culture provides an efficient approach for rapid multiplication of genetically uniform planting material throughout the year. However, successful establishment of aseptic cultures remains one of the major challenges in banana micropropagation, particularly when field-derived sucker explants are used.

Plant tissue culture relies fundamentally on maintaining strictly aseptic conditions during culture initiation, multiplication, and regeneration. Standard Murashige and Skoog (MS) basal medium, supplemented with sucrose, macro- and micronutrients, vitamins, and plant growth regulators, provides favorable conditions for plant growth as well as the proliferation of contaminating microorganisms. Bacteria, yeasts, and filamentous fungi associated with field-grown banana suckers may be introduced during explant excision and inoculation. These contaminants compete with plant tissues for nutrients, alter medium pH, release phytotoxic metabolites, inhibit shoot multiplication, and may ultimately result in culture deterioration and mortality.

Developing an effective surface sterilization protocol is therefore essential for successful in vitro establishment of *Musa acuminata* cv. Grand Naine (G-9). An ideal sterilization treatment should eliminate surface-associated microbial contaminants while maintaining the viability and regenerative potential of the explant. However, sterilization efficiency depends on several factors, including the type and concentration of disinfectant, exposure duration, explant size, physiological age, tissue characteristics, and initial microbial load. Insufficient disinfection may result in persistent microbial contamination, whereas excessive chemical exposure can damage meristematic tissues, induce phenolic exudation, cause tissue browning, and reduce explant survival.

Several chemical agents are commonly employed individually or in combination for plant tissue culture sterilization. Bavistin, containing carbendazim as its active ingredient, is a systemic fungicide commonly used as a preliminary treatment to suppress fungal contamination before surface sterilization. Ethanol acts as a rapid disinfectant by disrupting microbial membranes and denaturing proteins. Sodium hypochlorite (NaOCl) acts as an oxidizing agent through the formation of hypochlorous acid, which damages essential microbial cellular components. Mercuric chloride ($HgCl_2$) is a potent disinfectant with broad-spectrum antimicrobial activity through its interaction with microbial proteins. However, its toxicity and environmental persistence

require careful handling and appropriate hazardous-waste disposal.

Although these sterilizing agents are widely used in plant tissue culture, their effectiveness and phytotoxicity vary with plant species and explant type. Therefore, concentration and exposure duration must be optimized to achieve effective microbial elimination without compromising explant viability. The present study was undertaken to evaluate the comparative efficacy of different concentrations of Bavistin (1% to 5%) combination with ethanol 70%, mercuric chloride (0.1–0.5%), and sodium hypochlorite (1–5%) for the surface sterilization of banana sucker explants of *Musa acuminata* Colla cv. Grand Naine (G-9). The study aimed to identify an effective sequential sterilization protocol that minimizes microbial contamination while maintaining healthy explants suitable for subsequent in vitro culture initiation and micropropagation.

2. MATERIALS AND METHODS

2.1 Plant material and explant selection

Healthy, actively growing explants (suckers) of banana (*Musa acuminata* Colla) cv. Grand Naine (G-9)mother plant were selected for collection of suckers as the experimental starting material for in vitro culture initiation. Mother plants were maintained at field in Pachora Road Bilwadi and explants were harvested during September 2025 from juvenile, disease-free shoots free of visible insect damage or necrotic lesions. Prior to chemical treatments, excised explants were trimmed to uniform dimensions to minimize experimental variation arising from tissue size or differential surface area.

2.2 Preliminary washing and Bavistin pre-treatment

Freshly excised explants were initially washed thoroughly under running tap water for 15–20 min to dislodge adhering soil particles, dust, and superficial organic debris. Where required, a mild neutral liquid detergent (e.g., 2–3 drops of Tween-20 or Teepol per 100 mL) was included during gentle agitation, followed by repeated rinsing with distilled water until all traces of foam were removed. One layer of suckers removed after the rinsing with distilled water Subsequently, explants were subjected to a preliminary fungicidal pre-treatment using freshly prepared aqueous suspensions of commercial Bavistin at concentrations ranging from 1% to 5% (w/v) outside the laminar airflow cabinet to suppress fungal spores and superficial hyphae. Among the tested gradient (1–5%), a 2% Bavistin pre-treatment for 5 min provided the most satisfactory response without inducing visible tissue stress. The exact commercial formulation and active ingredient concentration (e.g., carbendazim 50% WP) should be documented from laboratory records. Following Bavistin immersion, explants were rinsed thoroughly 3–4 times with sterile distilled water to remove residual fungicide prior to transfer into the laminar airflow workstation.

2.3 Aseptic surface sterilization treatments and culture inoculation

All subsequent sterilization steps were performed under strictly aseptic conditions inside a pre-sterilized laminar airflow cabinet equipped with HEPA filtration. Surface sterilization treatments were evaluated across defined concentration ranges for each chemical agent: ethanol at 70%, (v/v); mercuric chloride ($HgCl_2$) at 0.1% (w/v); and sodium hypochlorite (NaOCl) at 3% (v/v). Based on experimental observations, the optimal exposure durations associated with maximum contamination control and tissue viability were 1 min for ethanol, 2 min for $HgCl_2$, and 10 min for sodium hypochlorite, with effective concentrations identified as 70% ethanol, 0.1% $HgCl_2$, and 3% sodium hypochlorite. During treatment, culture vessels containing explants and sterilant solutions were gently swirled at regular intervals to ensure uniform contact across all tissue surfaces and eliminate trapped air bubbles. Between consecutive chemical treatments and immediately following the final sodium hypochlorite exposure, explants were rinsed sequentially 3–5 times (2–3 min per rinse) with autoclaved double-distilled water to completely remove residual sterilants and prevent ongoing phytotoxicity. The exact number and duration of sterile water rinses, along with the basis of sodium hypochlorite concentration (commercial bleach dilution vs. available chlorine percentage), should be specified from the laboratory protocol.

After the final rinse, explants were blotted dry on sterile autoclaved filter paper, trimmed slightly at the cut ends to remove any chemically damaged basal cells, and aseptically inoculated vertically onto Half-strength Murashige and Skoog (MS) medium supplemented with carbon source, e.g., 3% (w/v) sucrose, gelling agent 0.8% (w/v) agar, phytohormones Indole-3-acetic acid (IAA) 2mg per liter and 6-Benzylaminopurine (6-BAP) 6 mg per liter, adjusted to pH 5.7–5.8 prior to autoclaving at 121 °C and 15 psi for 15 min. Inoculated culture bottles were maintained in a growth room at temperature, e.g., 25 ± 2 °C under a photoperiod16/8 h light/dark cycle with light provided by cool-white LED tub light at 3000 lux.

2.4 Experimental design

The experiment was structured to compare individual and combined sterilant concentration regimes for visible microbial contamination (fungal mycelial growth or bacterial oozing/turbidity), phenolic tissue browning, progressive tissue necrosis, and healthy green explant survival. Cultures were monitored regularly at 3-day intervals, over a total incubation period of 21–28 days.

The experiment was conducted using a Completely Randomized Design (CRD). Each treatment concentration was evaluated using three independent experimental replicates, with 10 explants per replicate. Thus, a total of 30 explants were used for each treatment concentration. The explants were distributed equally among the three replicates to ensure consistency and reliability of the experimental observations. The percentage of contamination and survival of explants were recorded for each treatment, and the results were evaluated based on the observations obtained from all three replicates.

3. EXPERIMENTAL OBSERVATION

3.1 Interpretation of Bavistin treatment results

Bavistin is a systemic fungicide used to control fungal contamination. Your results indicate a concentration-dependent response.

Concentration	Survival (%)	Contamination (%)	Browning/death (%)	Interpretation
1%	86.67	13.33	0.00	High survival, some contamination
2%	70.00	0.00	30.00	No contamination, good survival
3%	53.33	0.00	46.67	Reduced survival
4%	43.33	0.00	56.67	Increased tissue damage
5%	16.67	0.00	83.33	Severe tissue damage

3.2 Descriptive statistical analysis

The following table presents the total observations from three replicates (30 explants per treatment).

Treatment	Concentration	Surviving (Mean ± SD)	Contaminated (Mean ± SD)	Browned/Died (Mean ± SD)
T1	1%	8.67 ± 0.58	1.33 ± 0.58	0.00 ± 0.00
T2	2%	7.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.00
T3	3%	5.33 ± 0.58	0.00 ± 0.00	4.67 ± 0.58
T4	4%	4.33 ± 0.58	0.00 ± 0.00	5.67 ± 0.58
T5	5%	1.67 ± 0.58	0.00 ± 0.00	8.33 ± 0.58

Values represent mean ± standard deviation (SD), n = 3 replicates.

3.3 Percentage analysis

Treatment	Survival (%)	Contamination (%)	Browning/Death (%)
T1 (1%)	86.67	13.33	0.00
T2 (2%)	70.00	0.00	30.00
T3 (3%)	53.33	0.00	46.67
T4 (4%)	43.33	0.00	56.67
T5 (5%)	16.67	0.00	83.33

3.4 One-way ANOVA

One-way ANOVA was performed to determine whether the concentration treatments significantly affected explant survival.

ANOVA results

F-value
79.625
P-value
0.000000153
Degrees of freedom
4, 10
Significance level
0.05
Highly significant ($p < 0.001$)
Interpretation: The ANOVA indicates a statistically significant difference in explant survival among the five concentration treatments. Increasing the concentration resulted in a progressive reduction in explant survival.

4. RESULTS AND DISCUSSION

4.1 Results

The effect of different concentrations of Bavistin (1–5%) on explant contamination, survival and browning was evaluated using three replicates, with 10 explants in each replicate. A total of 30 explants were used for each treatment.

The results revealed considerable variation in explant survival and browning among the different Bavistin concentrations.

4.1.1 Effect of Bavistin concentration on explant survival

The highest explant survival (86.67%) was recorded in T1 (1% Bavistin), followed by T2 (2%) with 70% survival. A further increase in Bavistin concentration resulted in a progressive reduction in explant survival. T3 (3%) recorded 53.33% survival, while T4 (4%) showed 43.33% survival. The lowest survival percentage (16.67%) was observed in T5 (5% Bavistin).

These findings indicate that increasing Bavistin concentration negatively affected explant survival under the experimental conditions.

4.1.2 Effect of Bavistin concentration on contamination

Contamination was observed only in T1 (1% Bavistin), where 4 out of 30 explants (13.33%) showed contamination. No contamination was recorded in T2, T3, T4 or T5. The results indicate that Bavistin concentrations of 2% and above provided complete contamination control under the tested experimental conditions.

4.1.3 Effect of Bavistin concentration on explant browning and mortality

Browning and mortality increased with increasing Bavistin concentration. No browning or mortality was recorded in T1 (1%). However, T2 (2%) showed 30% browning and mortality, followed by 46.67% in T3 (3%).

The browning and mortality percentages further increased to 56.67% in T4 (4%) and 83.33% in T5 (5%). The maximum tissue damage was therefore observed at the highest Bavistin concentration.

4.1.4 Statistical analysis

One-way ANOVA revealed a highly significant difference in explant survival among the five Bavistin treatments ($F = 79.625$, p less than 0.001). The results indicate that Bavistin concentration had a significant influence on explant survival. Tukey's post-hoc test revealed significant differences between most treatment combinations, except between T3 (3%) and T4 (4%). The Kruskal–Wallis test also indicated a significant treatment effect ($H = 13.498$, $p = 0.0091$), supporting the observed differences in explant survival.

4.2 Discussion

Bavistin (Carbendazim) is a systemic benzimidazole fungicide commonly used for the management of fungal contamination. In plant tissue culture, fungicides may help control fungal contaminants associated with explants. However, their concentration and exposure duration are important factors influencing explant viability. In the present investigation, different concentrations of Bavistin showed variable effects on contamination control and explant survival. At 1% Bavistin, the highest survival percentage (86.67%) was recorded, although 13.33% contamination was observed. This suggests that the lower concentration was insufficient to eliminate all fungal contaminants associated with the explants. At 2% Bavistin, complete contamination control was achieved, with 70% explant survival. This concentration provided a better balance between contamination control and explant viability compared with the other treatments. However, increasing Bavistin concentrations to 3%, 4% and 5% resulted in a progressive decline in explant survival. The reduction in survival was accompanied by an increase in tissue browning and mortality. The highest concentration (5%) resulted in 83.33% browning and mortality, indicating a possible concentration-dependent phytotoxic response. The increased browning observed at higher concentrations may be associated with tissue stress, cellular damage and oxidation of phenolic compounds released from wounded explants. Nevertheless, the present experiment does not establish the precise biochemical mechanism responsible for browning. Further biochemical investigations would be required to confirm the underlying causes. The statistical analysis further supported the significant influence of Bavistin concentration on explant survival. The significant ANOVA result indicates that the observed differences among treatments were unlikely to be explained by random variation alone. However, the small number of replicates should be considered when interpreting these findings. Overall, the findings demonstrate that although higher Bavistin concentrations were effective in controlling visible contamination, they adversely affected explant survival. Therefore, increasing fungicide concentration beyond the effective level may not be beneficial for plant tissue culture establishment.

5.0 CONCLUSION

The present investigation revealed that Bavistin concentration plays an important role in controlling contamination and maintaining explant viability. Among the five concentrations evaluated, 1% Bavistin produced the highest survival (86.67%), whereas 2% Bavistin achieved complete contamination control with 70% survival.

Considering both contamination control and explant survival, 2% Bavistin emerged as the most promising treatment among the concentrations tested. Higher concentrations resulted in increased browning and mortality, suggesting possible phytotoxic effects.

6.0 RRFERENCES

1. Suman, S. (2017). Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. *Biochemical & Cellular Archives*, *17*(1), 1.
2. Strosse, H., Van den Houwe, I., & Panis, B. (2004). Banana cell and tissue culture-review. *Banana improvement: Cellular, molecular biology, and induced mutations*, 1-12.
3. Banerjee, N., & de Langhe, E. (1985). A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of Musa (banana and plantain). *Plant cell reports*, *4*(6), 351-354.
4. Kumar, P. D., Chakradhar, G. R., & Vimal, V. K. (2024). Tissue culture in banana cultivation: a review of its impact on disease management, yield improvement, and sustainable production. *Journal of Advances in Biology & Biotechnology*, *27*(9), 628-644.
5. Swamy, R. D., Rao, N. S., & Chacko, E. K. (1983). Tissue-culture propagation of banana. *Scientia Horticulturae*, *18*(3), 247-252.
6. Chukwu, S. C., Awala, S. K., Angombe, S., Valombola, J. S., Nanhapo, P. I., Mberama, C., ... & Musa, I. (2025). Recent progress in tissue culture techniques and biotechnological innovations for banana production (Musa spp.): a review. *Discover Plants*, *2*(1), 13.
7. Ngomuo, M., Mneney, E., & Ndakidemi, P. A. (2014). The in vitro propagation techniques for producing banana using shoot tip cultures. *American Journal of Plant Sciences*, *5*(11), 1614.
8. Erol, M. H., Dönmez, D., Biçen, B., Şimşek, Ö., & Kaçar, Y. A. (2023). Modern approaches to in vitro clonal banana production: Next-generation tissue culture systems. *Horticulturae*, *9*(10), 1154.
9. Muhammad, A., Hussain, I., Naqvi, S. S., & Rashid, H. (2004). Banana plantlet production through tissue culture. *Pakistan Journal of Botany*, *36*(3), 617-620.
10. Agbadje, E. T. A. E., Agbidinoukoun, A., Zandjanakou-Tachin, M., Cacaï, G. T., & Ahanhanzo, C. (2021). Mass production of bananas and plantains (Musa spp.) plantlets through in vitro tissue culture partway: A review. *European Journal of Biology and Biotechnology*, *2*(4), 1-8.

First Author

Dr. Vipul H. Phalak is an academician and researcher working as Assistant Professor in Biochemistry at Moolji Jaitha College (Autonomous), Jalgaon. He holds M.Sc. and Ph.D. degrees and has qualified NET, SET and GATE examinations. His areas of specialization include Plant Biotechnology, Plant Tissue Culture and Secondary Metabolites. His research focuses on plant tissue culture, seed dormancy, plant regeneration and biological activities of medicinal plants.