Plant Cell Culture & Micropropagation

http://pccm.ufla.br/index.php/plantcellculturemicropropagation/index

PPM™, Kasumin® and calcium hypochlorite in the disinfestation and *in vitro* introduction of *Nepenthes graciliflora* and *Dionaea muscipula*

Ana Clara Saraiva de Souza[1], André Oliveira Gomes e Silva[2], Nicolas Alexandre Gonçalo da Silva[2], Guilherme Jorge do Carmo Silva[1], Lailla Sabrina Queiroz Nazareno[3], Rogerio Luiz da Silva[4], Rogério Gomes Pêgo[2], Natane Amaral Miranda Padua[4]

ABSTRACT

Among the various genera of carnivorous plants, *Nepenthes* and *Dionaea* have gained prominence due to their greater diversity and their allelopathic, biological, medicinal, and pharmaceutical potential. Vegetative propagation of these genera offers advantages such as reduced production time, reproduction of important cultivars, and high multiplication potential. Thus, this study aimed to evaluate disinfestation methods and *in vitro* introduction of these two genera to enhance vegetative propagation. Nodal segments of *Nepenthes graciliflora* were used, testing three concentrations of calcium hypochlorite (0.75, 1.0, and 2.0%) for disinfestation and the presence and absence of PPM™ (Plant Preservative Mixture) in the nutritive medium. For *Dionaea muscipula*, rhizome sections were used, and we tested disinfection of donor plants with Kasumin® and the presence or absence of PPM™ in the culture medium. For disinfestation of N. graciliflora, PPM™ reduced the presence of microorganisms for up to 32 days of cultivation. Regarding calcium hypochlorite, a 2% concentration is recommended, with new tests needed to reduce contamination. For disinfestation of *D. muscipula* rhizomes, PPM™ resulted in the lowest contamination level, while Kasumin® provided the lowest oxidation rate. The combination of PPM™ and Kasumin® resulted in 60% sprouting of *D. muscipula* explants.

Keywords: Micropropagation; Carnivorous plants; Plant Preservative Mixture.

INTRODUCTION

Carnivorous plants can supplement their nutrition by digesting arthropods and even small animals through modified leaves (Adamec et al., 2021; Mithöfer, 2022). In Brazil, the commercialization and cultivation of these plants were restricted for many years to specific niches, such as plant collectors (Banasiuk et al., 2012). However, because of their landscaping potential, carnivorous plants have gained national prominence and become part of the flower and ornamental plant chain, a sector that grew by 9.95% in 2024 (CEPEA; IBRAFLOR, 2025). Internationally, their cultivation is already quite widespread, which is due to the fact that this group of plants produces secondary metabolites (Makowski et al. 2024) that are targets of studies by pharmaceutical industries for their medicinal potential (Wójciak et al., 2023; Su et al., 2024). Furthermore, studies have already shown that these metabolites have biocidal characteristics when used against insects (Rahman-Soad et al., 2021), monocotyledons, and eudicotyledons (Valente et al., 2022). Despite some differences among the genera of carnivorous plants, something they have in common is the fact that they grow in places where cultivation conditions are scarce regarding nutrients, and by capturing and using insects as a source of nutrition, they would have a great advantage over other plants,

https://doi.org/10.46526/pccm.2026.v21.216
Received in February 12, 2026 and approved in October 5, 2026
[1]Federal University of Lavras/UFLA, Lavras, MG, Brazil
[2]Department of Plant Science, Federal Rural University of Rio de Janeiro/UFRRJ, Seropédica, RJ, Brazil
[3]Graduate Program in Plant Science, Federal University of Lavras/UFLA, Lavras, MG, Brazil
[4]Department of Silviculture, Federal Rural University of Rio de Janeiro/UFRRJ, Seropédica, RJ, Brazil
*Corresponding author: anaclarasdsouza@gmail.com
Editor: Diego Ismael Rocha

which obtain most of their nutrients through the soil (Torre, 2023).

Two genera in this group are *Nepenthes*, which includes various species and hybrids, and *Dionaea*, which includes a single species, *D. muscipula*, popularly known as the "Venus flytrap" (Brewer and Schlauer, 2018). Their propagation can be done through cuttings and seeds in the case of *Nepenthes*, while *Dionaeas*, on the other hand, can be propagated by seeds, detached rhizomes, and leaves (Murthy et al., 2025).

Seeds are the traditional method of propagation for carnivorous plants; however, they are relatively slow, potentially taking several months to produce a single seedling (D' Amato, 2013; Murthy et al., 2025). Other factors also limit sexual propagation of these plants. The genus *Nepenthes* consists of dioecious plants, which depend on flowering synchronicity and environmental factors for successful crossing (Scharmann et al., 2019; Mao and Randayaphi, 2004). For *Dionaea muscipula*, which shows natural variation and distinct cultivars (D'Amato, 2013; Prawestri et al., 2024; Murthy et al., 2025), vegetative propagation is recommended to maintain its characteristics.

In vitro cultivation of vegetative propagules is an alternative to reduce production time and, compared with conventional methods, can generate numerous shoots per explant (Miguel et al., 2020; Dwiati et al., 2023; Bhattacharjee et al., 2024; Almeida et al., 2025). The application of tissue culture techniques for large-scale propagation can be promising, as these plants exhibit a high growth rate and vigorous development in a controlled environment, as well as a great potential for regeneration through their leaves (Murthy et al., 2025).

The first step in *in vitro* plant cultivation involves explant selection, disinfestation, and *in vitro* establishment (Miguel et al., 2020; Bhardwaj et al., 2025), and this stage is among the most challenging. Carnivorous plants can present certain difficulties in their introduction as they tend to be sensitive to sodium hypochlorite (Brewer and Schlauer, 2018), the most commonly used disinfectant in tissue culture. Furthermore, this group of plants contains a large number of endophytic agents, such as fungi and bacteria (Bhore et al., 2013; Naseem and Kayang, 2021), which can possibly be controlled by using specific antibiotics in the culture medium.

PPM™ is a cheaper alternative to conventional antibiotics because, at ideal doses, it is an extremely effective preservative/biocide that can reduce endogenous contamination (Plant Cell Technology, 2025). Other disinfesting substances, such as calcium hypochlorite and Kasumin®, may also reduce contamination in *in vitro*-inoculated plant materials, making evaluation of their effects important. Based on these considerations, this study aimed to evaluate the disinfestation and *in vitro* introduction of two carnivorous plant species, *Nepenthes graciliflora* and *Dionaea muscipula*, from vegetative propagules using different concentrations of calcium hypochlorite, PPM™, and Kasumin®.

MATERIAL AND METHODS

Nodal segments of *Nepenthes graciliflora* and rhizome sections of *Dionaea muscipula* were excised from mother plants initially cultivated in pots containing sphagnum moss, rice husks, and perlite, in a 2:1:1 ratio, and maintained in a greenhouse. For tissue disinfestation of both species, explants were first washed under running water using a soft brush. After initial cleaning, the explants were transferred to a Tween 20 solution (6 drops L^{-1}), where they remained under agitation for 10 minutes. Upon completing the agitation, the explants were washed three times with distilled water. Subsequent disinfestation steps were performed in a laminar flow hood, following the methodology of each proposed test.

The culture medium used was autoclaved MS® (Murashige and Skoog 1962), supplemented with 10 mL L^{-1} of Vitamin (1g L^{-1} Thiamine, 1g L^{-1} Pyridoxine, 1g L^{-1} Nicotinic Acid, and 0.4g L^{-1} Glycine) and 0.1 g L^{-1} Myo-Inositol, 25 g L^{-1} sucrose, and agar with pH adjusted to 5.7 ± 0.1. After inoculation, the material was maintained in a growth room at 25 ± 2ºC, with a 16 h photoperiod and a light intensity of 40 µmol m^{-2} s^{-1}; this cultivation environment was identical across all experiments.

Nepenthes graciliflora

Nodal segments of *Nepenthes graciliflora*, approximately 2 cm long and exhibiting a vegetative bud located just above the leaf, were used as explants. The explants were immersed in a Nativo® (systemic fungicide) solution at 2 mL L^{-1} for 30

minutes. Subsequently, they were washed 3 times in autoclaved distilled water and immersed in 70% alcohol for 1 minute. Then, they were placed in calcium hypochlorite solutions at concentrations of 0.75, 1.0, and 2.0% (w/v), supplemented with Tween 20 (4 drops per 100 mL), for 30 minutes. After this process, the explants were washed three times with autoclaved distilled water. Before inoculation, the darkened parts of the explant extremities were removed with a scalpel. The explants were inoculated into MS® culture medium at half concentration of macro- and micronutrients, supplemented with 2 g L^{-1} activated charcoal, 7 g L^{-1} agar, and tested for the absence and presence of 1 mL L^{-1} of PPM™ in the culture medium. Test tubes containing 10 mL of nutritive medium, sealed with cling film, were used. The tubes containing the explants were kept in complete darkness for 7 days before being transferred to the growth room.

A completely randomized design in a 3x2 factorial scheme (three concentrations of calcium hypochlorite and the presence and absence of PPM™ in the culture medium) with 10 replicates was used. During 65 days, contamination, oxidation, and the number of shoots emitted were evaluated.

Dionaea muscipula

D. muscipula plants were used, with a portion of them being subjected to the application of a 3 mL L^{-1} Kasumin® (systemic biocide) solution 72 hours before disinfestation. The plants were kept in a protected location without foliar wetting for 7 days before *in vitro* introduction. The rhizomes were removed, and their leaves were discarded before disinfestation. The rhizomes were submerged in a 2.5% (w/v) calcium hypochlorite solution supplemented with Tween 20 (6 drops L^{-1}). Subsequently, they were washed 3 times with autoclaved distilled water. The damaged extremities of the explants were removed with the aid of a scalpel, and then they were inoculated into MS® culture medium at half the concentration of macro- and micronutrients, supplemented with 1 g L^{-1} polyvinylpyrrolidone, 6.5 g L^{-1} agar, and tested for the presence or absence of 2 mL L^{-1} of PPM™. Test tubes containing 10 mL of nutritive medium and one explant per tube were used. The tubes containing explants were kept in complete darkness for 10 days and then transferred to the growth room.

A completely randomized design in a 2x2 factorial scheme (presence and absence of Kasumin® and PPM™) with 10 replicates was used. After 30 days, the percentages of contamination and oxidation, and the number of shoots emitted, were evaluated.

Data Analysis

The data were subjected to analysis of variance (ANOVA), and means were compared by Tukey's test at a 5% significance level, using R software version 4.3.3 (R Core Team, 2024).

RESULTS

Nepenthes graciliflora

PPM™ influenced the contamination rate of *Nepenthes graciliflora* nodal segments after 7, 15, and 32 days of cultivation. Regarding calcium hypochlorite use, as well as its interaction with PPM™, no significant differences ($p<0.05$) were observed (Table 1). At 65 days after inoculation, 100% of the explants showed contamination, regardless of the treatment.

Table 1: Results of the variance analysis for the *in vitro* contamination rate of *Nepenthes graciliflora* nodal segments at 7, 15, 32, and 65 days after inoculation, as a function of disinfestation with PPM™ and calcium hypochlorite: CV%: Coefficient of Variation, P: p-value probability, *: Significant at the 5%probability level ($p<0.05$), ns: Not significant ($p\geq0.05$).

	P			
DAYS	PPM™	Calcium hypochlorite	PPM™ x Calcium hypochlorite	CV%
7	0.0004*	0.4204^{ns}	0.1380^{ns}	58.97
15	0.0046*	0.1226^{ns}	0.5827^{ns}	41.95
32	0.0327*	0.1323^{ns}	0.1323^{ns}	25.25
65	0.3217^{ns}	0.3745^{ns}	0.3745^{ns}	0.00

Regarding oxidation and shooting rates of nodal segments, no significant differences were observed between treatments, and means for oxidation and shooting remained consistent throughout the evaluation period ($p\geq0.05$). For the shoot variable, no shoot emission was observed in the test up to the 15-day evaluation.

The use of PPM™, up to 32 days of the experiment, was a relevant factor for reducing the contamination rate of *Nepenthes graciliflora* nodal segments. The substance provided a delay in the

growth of fungi and bacteria in the culture medium (Table 2). At 7, 15, and 32 days, the treatments showed average contamination rates of 50%, 70%, and 86%, respectively. Treatments without PPM™, by contrast, showed average contamination rates of 90%, 96%, and 100%, respectively.

Table 2: Mean values for the in vitro contamination rate of *Nepenthes graciliflora* nodal segments at 7, 15, and 32 days after inoculation, as a function of the presence and absence of PPM™, independent of calcium hypochlorite concentrations. Means followed by different lowercase letters in the column are significantly different according to Tukey's test at a 5% significance level.

	Contamination(%)		
PPM™	7 days	15 days	32 days
0 mL L^{-1}	90[a]	96[a]	100[a]
1 mL L^{-1}	50[b]	70[b]	86[b]

For calcium hypochlorite treatments, no significant differences were observed in controlling contamination in *Nepenthes graciliflora* segments. The mean values for contamination, when evaluating the calcium hypochlorite factor, ranged between 60% and 100%, with all explants being contaminated by 65 days (Figure 1).

At 32 and 65 days, the presence of sprouting from vegetative buds in the nodal segments of *Nepenthes graciliflora* was observed (Figure 2). According to the F-test, the means for sprouting were not statistically different; at 32 days, they showed mean sprouting rates of 10% in the treatment without PPM™ and 0% in the treatment with PPM™, and at 65 days, they showed mean sprouting rates of 6% regardless of the treatment. In the 0.75, 1.0, and 2.0% calcium hypochlorite treatments, the average percentage of segments with shoots was 5, 0, and 15% at 32 days, and 5, 0, and 10% at 65 days, respectively.

Dionaea muscipula

of PPM™ use on oxidation, as did the biocide Kasumin®. The budding variable was influenced by the interaction between PPM™ and Kasumin® (Table 3).

PPM™ significantly reduced contamination of *D. muscipula* rhizome segments (Table 4). Mean contamination rates were 35% in the presence of PPMTM and 100% without PPM™. The results indicate that PPM™ in the culture medium significantly reduces microbial growth. Kasumin® in the explant donor plants did not affect contamination rates in the *in vitro* segments. Their mean contamination rates were 75% when Kasumin® was used and 60% when it was not.

For the oxidation percentage of *D. muscipula* explants, treatments with Kasumin® differed significantly ($p<0.05$) (Table 3). The averages indicate that Kasumin® reduced oxidation, with an average of 20% (Table 5). Treatments without Kasumin® showed 85% oxidation. Mean oxidation rates in the presence and absence of PPM™ did not differ significantly, at 60% and 45%, respectively.

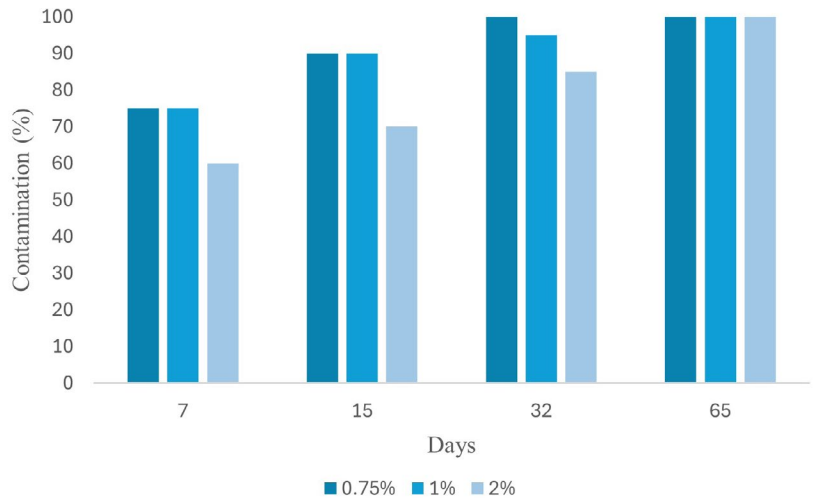


Figure 1: Mean values of *in vitro* contamination of *Nepenthes graciliflora* nodal segments at 7, 15, 32, and 65 days after inoculation, as a function of three concentrations of calcium hypochlorite (0.75, 1, and 2%).



Figure 2: Presence of sprouting from the vegetative bud present in the nodal segment of *Nepenthes graciliflora* at 65 days.

Table 3: Results of the variance analysis for the *in vitro* contamination, oxidation, and budding rates of *Dionaea muscipula* rhizomes, as a function of Kasumin® application and PPM™ use, at 42 days after inoculation. CV%: Coefficient of Variation, P: p-value probability, *: Significant at the 5%probability level ($p<0.05$), ns: Not significant ($p\geq0.05$).

	P			
Variable	PPM™	Kasumin®	PPM™ x Kasumin®	CV%
Contaminatio	0.000*	0.066^{ns}	0.059^{ns}	46.10
Oxidatio	0.285^{ns}	0.0000*	0.2372^{ns}	74.12
Prouting	0.000*	0.000*	0.000*	172.13

Table 4: Mean contamination values of *Dionaea muscipula* rhizomes inoculated *in vitro* as a function of PPM™ and Kasumin® use, after 42 days of inoculation. Means followed by different lowercase letters in the column are significantly different according to Tukey's test at a 5% significance level.

	CONTAMINATION (%)	
	PPM™	Kasumin®
Presence	35^{a}	75^{a}
Abcense	100^{b}	60^{a}

For the shoot variable, there was a significant interaction effect between the factors, where only with the combined use of both substances, Kasumin® and PPM™, was it possible to obtain shoots from the inoculated explants, with no such sprouting observed in treatments with the individual presence of the substances (Figure 3).

Table 5: Mean oxidation values of *Dionaea muscipula* rhizomes inoculated *in vitro* in response to the use of biocide Kasumin® and PPM™, after 42 days of cultivation. Means followed by different lowercase letters in the column are significantly different according to Tukey's test at a 5% significance level.

	OXIDATION %	
	PPM™	Kasumin®
Presence	60^{a}	20^{b}
Abcense	45^{a}	85^{a}

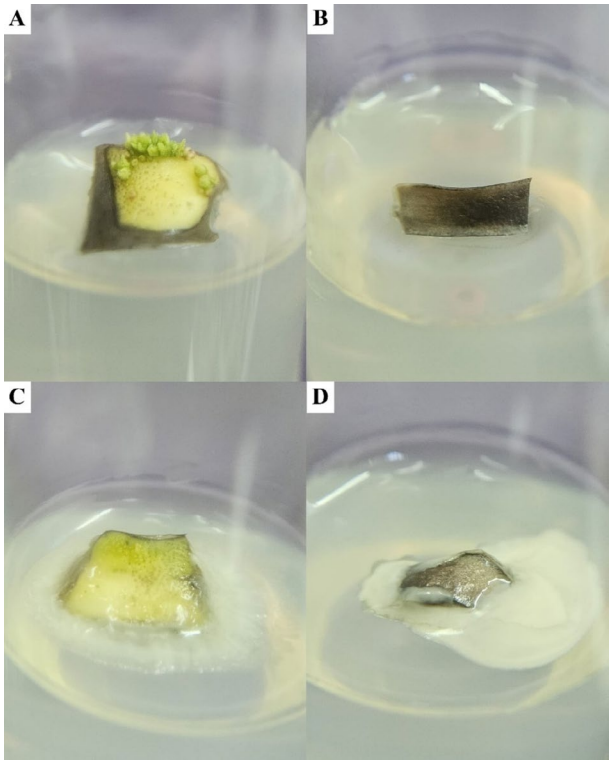


Figure 3: Shoot development in *Dionaea muscipula* explants, based on Kasumin® application and PPM™ use, at 42 days after inoculation: Treatment with PPM™ and Kasumin® showing shoots (A), Treatment with PPM™ and without Kasumin® without shoots (B), Treatment without PPM™ with Kasumin® without shoots (C), Treatment without PPM™ and without Kasumin® without shoots (D).

When rhizomes were treated with Kasumin® and inoculated into culture medium with PPM™, shoot formation occurred in 60% of the explants (Table 6).

Table 6: Mean budding values of *Dionaea muscipula* rhizomes inoculated *in vitro* as a function of the interaction between PPM™ and Kasumin® factors. Means followed by different lowercase letters in the column and different uppercase letters in the row differ significantly by Tukey's test at the 5% level of significance.

	PROUTING (%)	
PPM™	With Kasumin®	Without Kasumin®
Presence	60 aA	0 aB
Abcense	0 bA	0 aA

DISCUSSION

Several limitations affect the propagation of the genera *Nepenthes* and *Dionaea*. *Nepenthes* are dioecious plants (Scharmann et al., 2019; Lukmanasari et al., 2025), which hinders breeding because they do not exhibit sexual dimorphism. Consequently, identification is only possible at reproductive age, which can take five to seven years (Lukmanasari et al., 2025). Furthermore, flowering occurs at indeterminate times and periods and is influenced by plant nutrition and sunlight, as observed in other ornamental plants (Boulay et al., 2000; Cai et al., 2025; Wang et al., 2025). When seed production is successful, germination and initial development are extremely slow, potentially taking up to two months. Additionally, temperature and humidity influence the process (Mao and Ranyaphi, 2007). *Dionaea muscipula*, on the other hand, is monoecious (Hewitt-Cooper, 2016) and accepts self-pollination and cross-pollination. However, it exhibits various natural variations, as well as cultivars with distinct appearances (D'Amato, 2013), making vegetative propagation strictly recommended to maintain its characteristics (Murthy et al., 2025).

This group of plants also harbors endophytic agents, usually acquired over time, that often play an important role in plant development by acting symbiotically with the plant. However, the presence of microorganisms at any stage of micropropagation affects explant development. In other words, plants infected with fungi and bacteria have compromised multiplication and rooting, which can lead to death (Al Samir et al., 2026). These contaminants can be controlled by using specific antibiotics in the culture medium. Previous studies with pineapple (*Ananas comosus*) and *Staphylea pinnata* L. have demonstrated the use of antibiotics to control contamination (Leone et al., 2016; Szewczyk-Taranek et al., 2020). However, antibiotics can be a limiting factor. Selecting an antibiotic requires identifying the bacteria present within the explant, including whether they are Gram-positive or Gram-negative, for example. Furthermore, repeated antibiotic use can lead to bacterial resistance (Al Samir et al., 2026). Thus, initial management of donor plants and the use of broad-spectrum biocides in the culture medium can support disinfestation and explant introduction (Ram et al., 2024), serving as a viable alternative to antibiotics.

PPM™ is a broad-spectrum biocide used in plant tissue culture that can be added to the culture medium to reduce microbial contamination (Ram et al., 2024; Romadanova et al., 2022). Its effects on plant development may vary among species and culture conditions. In some cases, PPM™ can also reduce contamination associated with endophytic microorganisms (Ram et al., 2024; Romadanova et al., 2022).

For both genera, PPM™ proved efficient at reducing microbial contamination. The efficiency of the biocide in controlling microorganisms and its influence on *in vitro* growth has already been observed in other vegetatively propagated cultures, such as *Nilgirianthus ciliatus*, a vulnerable subshrub extensively exploited for its pharmacological properties. Nodal explants treated with a PPM™ pre-treatment, followed by inoculation in a medium also supplemented with the biocide, showed shoot regeneration with low contamination, suggesting that explant exposure time to PPM™ influences endophyte reduction (Ram et al., 2024). The efficiency of PPM™ is also observed in controlling bacterial contamination and its influence on the *in vitro* growth of sugarcane culture, where treatments with the biocide in culture medium were able to contain bacterial growth, with greater uniformity observed in seedling development using 0.75 mL L^{-1} (Digonzelli et al., 2005). Antibiotics can also be added to the culture medium to prevent contamination; however, conventional antibiotics are commonly associated with disadvantages such as low explant viability and reduced tissue regeneration potential (Tamošiūnė et al., 2022).

In addition to being added to the culture medium, PPM™ can also be used as an alternative disinfestation method. Compared with the conventional method using ethanol and sodium

hypochlorite, PPM™ influenced contaminant control and the *in vitro* growth of other vegetatively propagated crops, such as Andean blueberry (Moreno-Peña et al., 2025). When used with another control agent, such as Tagstin, it was also possible to eradicate endophytic agents (Vivek et al., 2025).

Calcium hypochlorite can be used for the disinfestation of carnivorous plant explants (Brewer and Schlauer, 2018); however, the treatments did not differ from each other in controlling the contamination of *Nepenthes graciliflora* segments. This fact may result from insufficient concentrations of the active ingredient used for disinfestation. Although calcium hypochlorite is widely used, as is sodium hypochlorite, its efficacy depends on factors beyond active ingredient concentration, such as explant size and the duration of disinfestation (Quynh et al., 2022). Although calcium hypochlorite and the presence of PPM™ reduce the contamination rate, new concentrations and combinations must be tested to reduce microorganism levels in the culture medium and extend the contamination-free period.

The percentage of browning of plant materials is, for the most part, possibly a consequence of the oxidation of phenolic compounds present in the explant tissues, due to the reaction of polyphenol oxidases, which produces dark exudates in the medium and browning of the explant (Abdalla et al., 2022). Therefore, the use of antioxidants or adsorbents in the culture medium can help control phenolic browning (Abdalla et al., 2022), allowing better *in vitro* plant development (Verde et al., 2021; Vrundha and Thomas, 2023).

Phenolization can contribute to explant oxidation, in which released phenols react with oxygen and may cause oxidative damage to plant cells (Abdalla et al., 2022). Phenol oxidation products can be phytotoxic and cause necrosis and, eventually, explant death (Abdalla et al., 2022). For the continuity of *in vitro* cultivation, it is essential that the plant material remains alive after its introduction. Carnivorous plants exude growth-inhibiting phenolic substances into the culture medium (Teng, 1999). This exudation also causes explant oxidation and, consequently, their death. Plumbagin has been identified as a major compound in *D. muscipula* extracts. These extracts and plumbagin showed phytotoxic effects on *Lactuca sativa* and *Allium cepa* in bioassays (Valente et al., 2022).

When analyzing the oxidation of *Nepenthes graciliflora* nodal segments, PPM™ use had no effect; in both its presence and absence, the average was 16.6%. When using PPM™ to introduce lateral buds of *Eucalyptus citriodora*, oxidation in the explants was also observed (Donato et al., 2007). In the test, the authors used PPM™ as a pre-treatment and also added it to the culture medium. Although pre-treatment with PPM™ solution was efficient in controlling contamination of *Eucalyptus citriodora*, an increase in culture medium oxidation was observed. Silva et al. (2021) also observed that oxidation can be seen in the presence of PPM™, when compared to the effect of its absence.

Some oxidation of *Nepenthes graciliflora* explants was also observed in treatments with 0.75, 1.0, and 2.0% calcium hypochlorite, with means of 15%, 10%, and 25%, respectively. The effects of calcium hypochlorite on contamination and subsequent plant development can vary with its concentration (Quynh et al., 2022), which reinforces the need to assess both disinfestation and explant response. In coconut embryo culture, Quynh et al. (2022) found that 0.5% calcium hypochlorite reduced contamination and favored shoot and root growth compared with the higher concentrations tested.

Studies show that overaccumulation of exudates by *D. muscipula* in the culture medium can compromise plant integrity (Teng, 1999). In *D. muscipula* leaf cultures, activated charcoal was required for bud initiation but was omitted after regeneration (Teng, 1999). Its effects therefore need to be considered according to the cultivation stage.

Pre-treatment of plant material before *in vitro* establishment can help reduce contamination (Ram et al., 2024; Romadanova et al., 2022). Shoot development in *Nepenthes graciliflora* was not influenced by the use of biocide, but for *D. muscipula*, the presence of PPM™ and the use of Kasumin® was decisive for shoot emission in explants. The results indicate that the morphogenic response can vary among species. When using PPM™ for wheat seed establishment, it was observed that increasing additions to the culture medium affected the development of germinated roots and shoots. This finding indicates that the concentration of PPM™ may affect plant development (Bošnjak Mihovilović et al., 2024).

PPM™ combined with Kasumin® was an important factor in shoot development in *D. muscipula*. This occurs because, depending on the concentration and the plant species introduced *in vitro*, PPM™ in the culture medium can affect shoot regeneration (Romadanova et al., 2022). Kushnarenko et al. (2022) reported similar results when using PPM™ for walnut micropropagation, where the addition of 0.2% (v/v) resulted in both reduced contamination and an increased number of shoots per explant.

CONCLUSION

Thus, we conclude that PPM™, calcium hypochlorite, and Kasumin® influence the success of the *in vitro* introduction phase of carnivorous plants from the *Nepenthes* and *Dionaea* genera. For disinfestation of Nepenthes graciliflora nodal segments, PPM™ and 2% calcium hypochlorite provide contamination control for 32 days. The use of PPM™ in the disinfestation of *Dionaea muscipula* rhizomes provides the lowest level of contamination when compared to the treatment without the biocide. Kasumin® application resulted in the lowest oxidation rate, and the combined use of PPM™ and Kasumin® aids shoot development from the introduced explant.

AUTHOR CONTRIBUTION

All authors contributed to the development, writing, and approval of this work. Ana Clara Saraiva de Souza conceived the basic idea, performed the experiments, and conducted statistical analyses. André Oliveira Gomes e Silva and Nicolas Alexandre Gonçalo da Silva assisted in the development of experiments and data collection. Rogerio Luiz da Silva, Rogério Gomes Pêgo, Guilherme Jorge do Carmo Silva, Lailla Sabrina Queiroz Nazareno and Natane Amaral Miranda Padua assisted in the writing and correction of the document.

REFERENCES

ABDALLA, N.; EL-RAMADY, H.; SELIEM, M. K.; et al. An academic and technical overview on plant micropropagation challenges. **Horticulturae**, v. 8, n. 8, article 677, 2022.

ADAMEC, L.; MATUŠÍKOVÁ, I.; PAVLOVIČ, A. Recent ecophysiological, biochemical and evolutional insights into plant carnivory. **Annals of Botany**, v. 128, n. 3, p. 241–259, 2021.

ALMEIDA, J. C. P.; DIAS, F. V.; CAETANO, P. A. S. A técnica de micropropagação *in vitro* como estratégia de inovação tecnológica na produção de mudas. **Revista Gestão & Sustentabilidade**, v. 7, n. 1, article e15043, 2025.

AL SAMIR, E. A.; ALBANDAR, I.; KADHIM, M. J.; et al. The role of nanotechnology in the control of bacterial contamination in plant tissue culture: a review. **Al-Kunooze Scientific Journal**, v. 12, n. 1, p. 61–74, 2026.

BANASIUK, R.; KAWIAK, A.; KRÓLICKA, A. *In vitro* cultures of carnivorous plants from the *Drosera* and *Dionaea* genus for the production of biologically active secondary metabolites. **BioTechnologia**, v. 93, n. 2, p. 87–96, 2012.

BHARDWAJ, S.; KUMARI, M.; CHETHAN, T.; et al. Advances in micropropagation and tissue culture for horticultural crops: a review. **Plant Cell Biotechnology and Molecular Biology**, v. 26, n. 11–12, p. 166–182, 2025.

BHATTACHARJEE, S.; WASHMIN, N.; BORAH, T.; et al. Conspectus on endangered carnivorous pitcher plant *Nepenthes khasiana* Hook.f. emphasizing *in vitro* regeneration, pitcher development, and stability in genetic makeup. **South African Journal of Botany**, v. 167, p. 270–284, 2024.

BHORE, S. J.; KOMATHI, V.; KANDASAMY, K. I. Diversity of endophytic bacteria in medicinally important *Nepenthes* species. **Journal of Natural Science, Biology and Medicine**, v. 4, n. 2, p. 431–434, 2013.

BOŠNJAK MIHOVILOVIĆ, A.; KEREŠA, S.; LAZAREVIĆ, B.; et al. The use of sodium hypochlorite and plant preservative mixture significantly reduces seed-borne pathogen contamination when establishing *in vitro* cultures of wheat (*Triticum aestivum* L.) seeds. **Agriculture**, v. 14, n. 4, article 556, 2024.

BOULAY, J.; LETILLOIS, B.; LEGENDRE, L. Les *Nepenthes*. **Acta Botanica Gallica**, v. 147, n. 1, p. 97–110, 2000.

BREWER, J. S.; SCHLAUER, J. Biogeography and habitats of carnivorous plants. In: ELLISON, A. M.; ADAMEC, L. (eds.). Carnivorous plants: physiology, ecology, and evolution. Oxford University Press, 2018. p. 7–21.

CAI, Z. R.; SUKMA, D.; CHAN, M. T. Balancing nature and technology: enhancing flowering in ornamental plants. **Botanical Studies**, v. 66, article 41, 2025.

CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA (CEPEA); INSTITUTO BRASILEIRO DE FLORICULTURA (IBRAFLOR). Relatório PIB da cadeia de flores e plantas ornamentais brasileira: 2024. CEPEA/ESALQ-USP; IBRAFLOR, 2025. Available at: https://www.cepea.org.br/upload/kceditor/files/Relat%C3%B3rio%20Flores%20e%20plantas%20ornamentais%20-%202024(2).pdf. Accessed in: February 1, 2026.

D'AMATO, P. The savage garden: cultivating carnivorous plants. Ten Speed Press, 2013. 384p.

DIGONZELLI, P.; DÍAZ, L.; CARRIZO DE BELLONE, S. Uso de PPM (Plant Preservative Mixture) para controlar contaminantes bacterianos en la multiplicación *in vitro* de caña de azúcar. **Revista de la Facultad de Agronomía**, v. 22, n. 1, p. 23–33, 2005.

DWIATI, M.; WIDODO, P.; SUSANTO, A. H. Shoot regeneration in *Nepenthes mirabilis* as affected by flurprimidol and GA3 application. **Biodiversitas**, v. 24, n. 7, p. 4168–4174, 2023.

HEWITT-COOPER, N. Carnivorous plants: gardening with extraordinary botanicals. Timber Press, 2016. 232p.

KUSHNARENKO, S.; ARALBAYEVA, M.; RYMKHANOVA, N.; et al. Initiation pretreatment with Plant Preservative Mixture™ increases the percentage of aseptic walnut shoots. ***In Vitro* Cellular & Developmental Biology – Plant**, v. 58, p. 964–971, 2022.

LEONE, G. F.; ALMEIDA, C. V.; ABREU-TARAZI, M. F.; et al. Antibiotic therapy in pineapple (*Ananas comosus*) microplants. **Ciência Rural**, v. 46, n. 1, p. 89–94, 2016.

LUKMANASARI, P.; PURWANTORO, A.; SULISTYANINGSIH, E.; et al. Sex determination in *Nepenthes sumatrana* (Miq.) Beck based on morphological traits and molecular markers. **SABRAO Journal of Breeding and Genetics**, v. 57, n. 4, p. 1545–1555, 2025.

MAKOWSKI, W.; MRZYGŁÓD, K.; SZOPA, A.; et al. Effect of agitation and temporary immersion on growth and synthesis of antibacterial phenolic compounds in genus *Drosera*. **Biomolecules**, v. 14, n. 9, article 1132, 2024.

MAO, A. A.; RANYAPHI, R. A. Seed viability studies in *Nepenthes khasiana* Hook. f.: comparison of *in vivo* and *in vitro* seed germination. **Nelumbo**, v. 49, n. 1–4, p. 89–94, 2007.

MIGUEL, S.; MICHEL, C.; BITEAU, F.; et al. *In vitro* plant regeneration and Agrobacterium-mediated genetic transformation of a carnivorous plant, *Nepenthes mirabilis*. **Scientific Reports**, v. 10, article 17482, 2020.

MITHÖFER, A. Carnivorous plants and their biotic interactions. **Journal of Plant Interactions**, v. 17, n. 1, p. 333–343, 2022.

MORENO-PEÑA, L.; HIDALGO-ESCOBAR, K.; CEVALLOS-CEVALLOS, J. M.; et al. An effective disinfection protocol for contamination control *in vitro* establishment of Mortiño (*Vaccinium floribundum* Kunth) and identification of endogenous microbes. **Scientia Agropecuaria**, v. 16, n. 3, p. 375–384, 2025.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum**, v. 15, n. 3, p. 473–497, 1962.

MURTHY, H. N.; NAGELLA, P.; JOSEPH, K. S.; et al. The micropropagation of carnivorous plants: an overview. **Plant Cell, Tissue and Organ Culture**, v. 162, article 76, 2025.

NASEEM, F.; KAYANG, H. Endophytic fungal diversity of endemic carnivorous plant *Nepenthes khasiana* in Meghalaya, India. **Studies in Fungi**, v. 6, n. 1, p. 138–150, 2021.

PRAWESTRI, A. D.; RAHAYU, R. S.; KURNIAJATI, W. S.; et al. *In vitro* seed germination and shoot growth of *Nepenthes jamban* Chi. C. Lee, Hernawati & Akhriadi, a unique pitcher plant from Indonesia. **Journal of Tropical Biodiversity and Biotechnology**, v. 9, n. 2, article jtbb87674, 2024.

QUYNH, T. P.; XUAN, H. T. L.; MY, T. A.; et al. Effect of calcium hypochlorite on surface sterilization and seedling growth of Vietnamese coconut varieties. **Vietnam Journal of Biotechnology**, v. 20, n. 4, p. 663–673, 2022.

RAHMAN-SOAD, A.; DÁVILA-LARA, A.; PAETZ, C.; et al. Plumbagin, a potent naphthoquinone from *Nepenthes* plants with growth inhibiting and larvicidal activities. **Molecules**, v. 26, n. 4, article 825, 2021.

RAM, J. P. S.; RAMAKRISHNAN, R.; KUMAR, K. P.; et al. Phyto-safe *in vitro* regeneration and harnessing antimicrobial-resistant endophytes as bioinoculants for enhanced growth and secondary metabolites yield in *Nilgirianthus ciliatus*. **BMC Plant Biology**, v. 24, article 872, 2024.

R CORE TEAM. R: a language and environment for statistical computing. R Foundation for Statistical Computing, 2024. Available at: https://www.R-project.org/. Accessed in: September 25, 2026.

ROMADANOVA, N. V.; TOLEGEN, A. B.; KUSHNARENKO, S. V.; et al. Effect of Plant Preservative Mixture™ on endophytic bacteria eradication from *in vitro*-grown apple shoots. **Plants**, v. 11, n. 19, article 2624, 2022.

SCHARMANN, M.; GRAFE, T. U.; METALI, F.; et al. Sex is determined by XY chromosomes across the radiation of dioecious *Nepenthes* pitcher plants. **Evolution Letters**, v. 3, n. 6, p. 586–597, 2019.

SILVA, A. C. L.; MANFIO, C. E.; LEÃO, J. R. A.; et al. Indução da calogênese em segmentos foliares de seringueira (*Hevea* spp.) na Amazônia Sul-Ocidental. **Research, Society and Development**, v. 10, n. 9, article e17410917639, 2021.

SU, H. H.; LIN, E. S.; HUANG, Y. H.; et al. Inhibition of SARS-CoV-2 Nsp9 ssDNA-binding activity and cytotoxic effects on H838, H1975, and A549 human non-small cell lung cancer cells: exploring the potential of *Nepenthes miranda* leaf extract for pulmonary disease treatment. **International Journal of Molecular Sciences**, v. 25, n. 11, article 6120, 2024.

SZEWCZYK-TARANEK, B.; JAGLARZ, A.; PAŁKA, P.; et al. Identification and control of endophytic bacteria during *in vitro* cultures of *Staphylea pinnata* L. **Folia Horticulturae**, v. 32, n. 1, p. 47–55, 2020.

TAMOŠIŪNĖ, I.; ANDRIŪNAITĖ, E.; VINSKIENĖ, J.; et al. Enduring effect of antibiotic Timentin treatment on tobacco *in vitro* shoot growth and microbiome diversity. **Plants**, v. 11, n. 6, article 832, 2022.

TENG, W. L. Source, etiolation and orientation of explants affect *in vitro* regeneration of Venus fly-trap (*Dionaea muscipula*). **Plant Cell Reports**, v. 18, n. 5, p. 363–368, 1999.

TORRE, D. Carnivorous plants. Reaktion Books, 2023. 224p.

VALENTE, P. M.; VALENTE, V. M.; SILVA, M. C.; et al. Phytotoxicity and cytogenotoxicity of *Dionaea muscipula* Ellis extracts and its major compound against *Lactuca sativa* and *Allium cepa*. **Biologia**, v. 77, n. 10, p. 2975–2988, 2022.

VERDE, D. D. S. V.; SOUZA, M. M. I.; SILVA, A. S.; et al. Ácido ascórbico e polivinilpirrolidona no cultivo *in vitro* de *Dioscorea* spp. **Research, Society and Development**, v. 10, n. 9, article e10510917812, 2021.

VIVEK, A. S.; NADIYA, F.; JINSHA, P. T. A robust approach for eradication of endophyte and multiple shoot induction of *Piper barberi* Gamble: an endemic and endangered species of Southern Western Ghats. **Plant Cell, Tissue and Organ Culture**, v. 160, n. 3, article 76, 2025.

VRUNDHA, C. P. K.; THOMAS, T. D. Control of media browning during micropropagation and assessment of biochemical and clonal fidelity of *in vitro*-derived and mother plants in *Thottea siliquosa* (Lamk.) Ding Hou., an important ethnomedicinal shrub. **Journal of Genetic Engineering and Biotechnology**, v. 21, article 70, 2023.

WANG, J.; WANG, Q.; GAO, J.; et al. Genetic regulatory pathways of plant flowering time affected by abiotic stress. **Plant Stress**, v. 15, article 100747, 2025.

WÓJCIAK, M.; FELDO, M.; STOLARCZYK, P.; et al. Biological potential of carnivorous plants from Nepenthales. **Molecules**, v. 28, n. 8, article 3639, 2023.