

DOI: <https://doi.org/10.24297/jaa.v10i0.8282>**The Stimulating Effects of Imazalil and Carbendazim Fungicides on *In Vitro* Propagation and *Ex Situ* Conservation of The Medicinal Balkan Range-Restricted *Sideritis Raeseri* Boiss & Heldr. Subsp. *Raeseri***

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Abstract

Sideritis raeseri Boiss & Heldr. subsp. *raeseri*, known in Greece as Mountain tea of Parnassus or Velouchi is a range restricted medicinal plant of the Balkan peninsula. Conventional propagation methods did not allow the mass production of plant material in a short period of time due to both low seed germination rate and rooting of cuttings. Therefore, the aim of this study was to establish a reliable, reproducible and efficient regeneration protocol for mass and large-scale micropropagation, germplasm and *ex situ* conservation of *S. raeseri* Boiss & Heldr. using Imazalil and Carbendazim fungicides. After 9 weeks, 2.5-10 mg/l Imazalil stimulated root length by 1 cm but diminished root number and rooting percentage. Optimum shoot number (5.58), shoot length (24.91 mm), shoot multiplication (100%), root number (20.63) and rooting (66.67%) were recorded with 0.5 mg/l kinetin (KN) + 0.05 mg/l α -naphthaleneacetic acid (NAA) (Imazalil-free). After 4 ½ weeks, 1 mg/l Carbendazim + 0.5 mg/l 6-benzyladenine (BA) yielded 5.77 shoots/explant, 16.1 mm shoot length and 100% shoot multiplication. However, Carbendazim did not result in rooting. The *ex vitro* survival percentage of rooted shoot-tip explants derived from Imazalil experiment was 95%. Carbendazim proved useful in shoot proliferation and Imazalil in root elongation of *S. raeseri* Boiss & Heldr. subsp. *raeseri* micropropagation system.

Indexing terms/Keywords: benzimidazoles, carbendazim, fungicides, mountain tea of Parnassus or Velouchi, imazalil, imidazoles, micropropagation, *Sideritis raeseri* Boiss. & Heldr. subsp. *raeseri*, vitrification.

Introduction

Sideritis L. (Family Lamiaceae) comprises approximately 150 species of annuals and perennials distributed throughout North Africa, the Iberian Peninsula, the Mediterranean countries, and the Middle East region [1]. In Greece, *S. raeseri* Boiss. & Heldr. (Mountain tea of Velouchi or Parnassus) is distributed in central, north-central and northern parts of the country (typically in the range of Pindos) or in neighbouring areas with Albania and the FYR of Macedonia according to Strid and Kit Tan [2]. *S. raeseri* L. is a perennial herb of up to 40 cm, which self-sows in Parnassus, Timfristos (Velouchi) and other mountains of Aetolia, Doris and Fthiotida [3]. Aerial flowering parts of plants from *Sideritis* genus are known as "mountain tea," which is widely used in Mediterranean folk medicine as a very popular tea because of its anti-inflammatory [4], carminative, analgesic, antitussive, stomachic, and antimicrobial properties [5].

The application of fungicides in *in vitro* culture has shown that some of these substances may exhibit organogenic and morphogenic effects on *in vitro* plants. Thus, it has been discovered that the imidazole fungicides, such as imazalil either intensify the effect of exogenous cytokinins or inhibit biosynthesis of the gibberellic acid. A number of interesting side effects of the imidazole fungicide Imazalil can be observed in diverse tissue cultured plant species, i.e. excessive shoot formation in *Araceae*, inhibition of bushiness in *Gerbera*, restoration of normal embryo development in *Citrus* and histogenic instability of *Ficus benjamina* L. (Moraceae) chimeras [6]. When screening fungicides as potential tissue culture medium additives to control fungal development, it was found that the imidazole fungicide Imazalil exuberantly enhanced the shoot (bud)-inducing effect of BA in *Spathiphyllum floribundum*. On cytokinin-free medium imazalil did not induce buds [7]. Since the

past decade, some azole derivatives, such as imazalil have been found promising to enhance the shoot inducing capacity of different cytokinins, including TDZ. According to Werbrouck and Debergh [8], TDZ mediated shoot proliferation improved by the inclusion of Imazalil in *S. floribundum* culture.

Bavistin is a systemic fungicide that belongs to benzimidazole family containing 50% WP carbendazim. Benzimidazole is a group of organic fungicides with systemic action that are extensively used in agriculture [9]. It has been reported that the molecular structure of methyl benzimidazole carbonate has some resemblance to cytokinins based on adenine [10], showing strong cytokinin-like activity in direct shoot regeneration. Earlier studies have shown that bavistin enhances differentiation of roots and shoots in calli derived from carrot segment [10]. In addition, the shoot regeneration promoting activity of bavistin resulted in increased biosynthesis of endogenous cytokinins within the cultured explants [11].

Plant tissue culture (PTC) offers a viable alternative method of propagation. It is used widely in the conservation of rare, endangered and medicinal plants and also commercial propagation. PTC constitutes a way to maintain available competent explants and free from contamination for use *in vitro* propagation and genetic transformation, besides being highly convenient for conservation of *in vitro* germplasm and cryopreservation [12]. PTC in turn is the only way to sustain the large-scale farming of medicinal plants, as this is the only technique to produce plants of high and uniform quality in large quantity from any part of the plant in any season [13].

Sideritis plants in the wild are sexually and asexually propagated [14]. Micropropagation was considered necessary for *S. raeseri* bulk vegetative propagation as the nature of the plant is such that does not give a large number of cuttings. The biggest problem is observed during rooting and acclimatization of the plantlets produced. At the same time, it will be the first results obtained with *Sideritis* spp. species using fungicides as micropropagation enhancing agents. Aiming at the improvement and advancement of shoot and root direct regeneration *in vitro* and bearing in mind the activity of imidazole and benzimidazole fungicides, all these aspects orientated us towards discovering their effect on *in vitro* shoot multiplication and rooting by developing a large-scale propagation protocol through direct plant regeneration using shoot tip explants. Therefore, the obtained results of this study and the final established micropropagation protocol for *S. raeseri* are going to be novel and of sufficient biotechnological importance.

Materials and methods

Plant material and culture conditions

The experimental material was shoot tip explants from previous *S. raeseri in vitro* cultures. For the initial establishment of the plant material *in vitro*, apex meristems were cut and removed from the mother plants maintained in a peat: perlite (1:1) substrate in pots under unheated-greenhouse conditions. For the disinfection of the collected plant material, shoot tips were soaked in 70% ethanol for 1 min followed by 2% NaOCl solution for 15 min under continuous stirring. The successfully established explants were sub-cultured every 4 weeks until a sufficient amount of plant material to be concentrated. Two experiments were conducted. The nutrient medium used was the MS [15]. The pH of the medium in both experiments was adjusted to 5.8 before adding the gelling agent and afterwards the medium was sterilized at 121 °C for 20 min. For the experiment, shoot tip explants (1-2.5 cm, long) were transferred into Magenta vessels (Baby food jars, 62.4 mm × 95.8 mm, 200 ml) containing 35 ml of MS culture medium. All cultures were maintained in a growth chamber. The chamber was programmed to maintain a 16-h light duration (40 μmol/m²/s) supplied by cool white fluorescent lamps and a constant temperature of 22 ± 2 °C.

Experiment 1: Effect of Imazalil on micropropagation of *S. raeseri*

Imazalil (Fluka, PESTANAL®, analytical standard) was applied exogenously at 5 concentrations (0, 1, 2.5, 5, 10 mg/l) in combination with 0.5 mg/l KN (Sigma-Aldrich) and 0.05 mg/l NAA (Sigma-Aldrich). The MS culture medium was supplemented with 30 g/l sucrose (Duchefa) and 8 g/l agar (Sigma-Aldrich) as a gelling agent. The experiment included 5 treatments with 12 replications (explants)/treatment and 4 shoot tip explants in each

vessel. After 9 weeks of culture, measurements were taken regarding shoot number/explant, shoot length (mm), shoot multiplication percentage (%), root number/rooted microcutting, root length (mm), rooting percentage (%), callus formation percentage (%), vitrification and necrosis percentages (%).

Experiment 2: Effect of Carbendazim on micropropagation of *S. raeseri*

Carbendazim (97%, Sigma-Aldrich) was applied exogenously at 10 concentrations (0, 1, 2.5, 5, 10, 25, 50, 100, 200, 400 mg/l) in combination with 0.5 mg/l BA (Sigma-Aldrich). The culture medium was supplemented with 30 g/l sucrose (Duchefa) and 3 g/l Gelrite (Duchefa) as a gelling agent. The experiment included 10 treatments with 15 replications/treatment and 3 shoot tip explants in each vessel. After 4½ weeks of culture, data were recorded in terms of shoot number/explant, shoot length (mm) and percentages (%) of shoot multiplication, callus formation and vitrification.

Ex vitro acclimatization of in vitro rooted plantlets

Plantlets with well-developed shoots and roots were removed from the glass test tubes, washed thoroughly with tap water and transferred to an enriched peat (Terrahum): perlite (Perflor) (1:1 v/v) soil substrate. The rooted microplantlets were transferred to multi-point discs. Then, these trays were placed in a nylon table bench tunnel with adjustable relative humidity or misting system, initially, in the first week with 65-72% relative humidity and the second week with 55-62%. After two weeks the trays with the plants were transferred to one of the benches of the greenhouse (50 ± 5% relative humidity) for two more weeks, wherein watered by sprinkling. After this period, the plants were transplanted into pots of 0.5L, transferred to the nursery outside greenhouse, where the acclimatization of the plants was completed. After 8 weeks from transplantation, the adjusted plants were transferred to pots of larger capacity, 1.5 L, filled with enriched white peat moss (TS2, Clammann): perlite (Perflor): sand (2:2:0.5 v/v) soil substrate and maintained in the greenhouse. Finally, after 12 weeks from the initial transition of the *in vitro* rooted plantlets to the *ex vitro* environment, their survival percentage was recorded.

Statistical analysis

The experiment was completely randomized and analysed by ANOVA (Analysis of Variance) using the statistical program SPSS 17.0 (SPSS Inc., Illinois, New York, USA) at $P \leq 0.05$, according to Duncan's multiple range test ± Standard Error (S.E.) in order significant differences among the treatments to be established.

Results and discussion

Experiment 1: Effect of Imazalil on micropropagation of *S. raeseri*

In the current study employing *S. raeseri*, imazalil (1-10 mg/l) significantly reduced shoot number/explant from 5.58 (control) to 2.17-3 as well as shoot multiplication percentage by half, from 100% in the control treatment to 41.67-58.33%. Moreover, Imazalil at 10 mg/l caused 1 cm - decrease in shoot length (Table 1). Therefore, in *S. raeseri* Boiss & Heldr, Imazalil exerted an inhibitory effect on shoot regeneration. On the contrary, Imazalil improved the efficacy of TDZ in *S. floribundum* [8] and this enhancing effect of Imazalil was attributed to its effect on changing the metabolism of exogenously applied cytokinins. Imazalil was also reported to have an inhibitory effect on cytokinin degrading enzymes [7, 16]. In the present study with *S. raeseri*, shoot number/explant (5.58), shoot length (24.91 mm) and shoot multiplication percentage (100%) were maximum in the control combination treatment, 0.5 mg/l KN + 0.05 mg/l NAA, in devoid of Imazalil (Table 1 and Fig. 1a). On the other hand, in *S. floribundum* "Petite" Schott, multiple shoot induction was significantly increased by using Imazalil [7]. The stimulating effect of Imazalil in shoot proliferation of *S. floribundum* was recently stated to be due to inhibition of gibberellin (GA) biosynthesis [9]. Our results are also not in line with those reported by Tefera and Wannakraioj [17] for korarima [*Aframomum corrorima* (Braun) Jansen] where 4 mg/l Imazalil + 0.1 mg/l BA raised shoot number. According to Wannakraioj and Tefera [18], in Siam cardamom (*Amomum krervanh* Pierre ex Gagnep.), high axillary shoot proliferation and shoot growth rate were attained using a

modified MS medium supplemented with 0.5 mg/l TDZ + 2 mg/l Imazalil. Therefore, the different response of studied *S. raeseri* to Imazalil treatments regarding shoot number seems to be genotype- and concentration-dependent.

Table 1. Effect of Imazalil concentration (0-10 mg/l) combined with 0.5 mg/l KN and 0.05 mg/l NAA on shoot number/ explant, shoot length (mm), shoot multiplication, vitrification and necrosis percentages (%) in *S. raeseri* Boiss & Heldr. subsp. *raeseri*.

Imazalil (mg/l)	Shoot number / explant	Shoot length (mm)	Shoot multiplication (%)	Vitrification (%)	Necrosis (%)
Control	5.58 ± 0.75 b	24.91 ± 2.41 b	100 d	16.67 c	0 a
1	3.00 ± 0.85 a	25.99 ± 4.89 b	50 b	25 d	25 c
2.5	3.00 ± 0.69 a	17.15 ± 1.39 ab	58.33 c	0 a	16.67 b
5	2.83 ± 0.66 a	21.18 ± 3.16 ab	58.33 c	8.33 b	0 a
10	2.17 ± 0.60 a	15.80 ± 0.96 a	41.67 a	0 a	0 a
P-values	0.016*	0.060 ns	0.000***	0.000***	0.000***

Means (n=12) ± standard error (S.E) with the same letter in each column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$. Ns $P \geq 0.05$; * $P \leq 0.05$; *** $P \leq 0.001$.

Shoot multiplication percentage of *S. raeseri* was diminished due to Imazalil application. In contrast, the optimum shoot multiplication rate for korarima was obtained with 2 mg/l Imazalil + 0.5-0.75 mg/l TDZ [19]. Similarly, to our results, 12.5-50 μM Imazalil did not affect to a large extent the multiplication rate in Gerbera 'Rosabella' (bushy) cultivar, however, higher Imazalil concentrations (100-200 μM) led to cessation of shoot development [20]. In the Gerbera 'Sunset' (non-bushy) cultivar, Imazalil (12.5-25 μM) did not affect the multiplication rate although 50 μM Imazalil caused a slight, but significant reduction in shoot number. Imazalil at high concentrations (100-200 μM) acted as a growth retardant leading to bushy shoots, complete inhibition of shoot development, necrotic symptoms and subsequent death of explants [20]. A decrease in shoot length of *S. raeseri* explants was recorded, approximately by 1 cm due to Imazalil application at 10 mg/l, indicating that Imazalil at high concentrations is implicated in GA biosynthesis. As reviewed by Fletcher et al. [21], certainazole compounds, such as Imazalil interfere with the biosynthesis of gibberellins and influence plant morphogenesis, indicating their possible function as plant bio-regulators. The mode of action of Imazalil is unclear; it only induces shoots in the presence of exogenous cytokinins such as BA, indicating that Imazalil might alter BA metabolism or catabolism [6]. The percentage of vitrified *S. raeseri* explants was ranged between 0 and 25%. Necrotic symptoms were observed to the 16.67-25% of the explants treated with 1 or 2.5 mg/l Imazalil whereas no necrosis occurred to the Imazalil-untreated explants and explants treated with 5 or 10 mg/l Imazalil (Table 1).

In this research study with *S. raeseri* microplants, Imazalil reduced root number from 20.63 in the control treatment (Fig. 1a) to 6.71-10 (2 to 3 times decrease) (Fig. 1b-1e). On the other hand, root length was enhanced (31.95-35.72 mm) when explants treated with 2.5-10 mg/l Imazalil (Table 2).

Table 2. Effect of Imazalil concentration (0-10 mg/l) combined with 0.5 mg/l KN and 0.05 mg/l NAA on root number/ rooted microcutting, root length (mm), rooting and callus formation percentages (%) in *S. raeseri* Boiss & Heldr. subsp. *raeseri*.

Imazalil (mg/l)	Root number/ rooted microcutting	Root length (mm)	Rooting (%)	Callus formation (%)
Control	20.63 ± 2.84 b	23.87 ± 1.32 a	66.67 c	100 d
1	9.29 ± 0.97 a	22.07 ± 2.06 a	66.67 c	100 d
2.5	6.71 ± 0.64 a	31.95 ± 3.47 b	58.33 b	25 a
5	10.00 ± 1.40 a	33.14 ± 2.28 b	58.33 b	83.33 c
10	8.50 ± 0.55 a	35.72 ± 2.09 b	25 a	50 b
P-values	0.000***	0.000***	0.000***	0.000***

Means (n=12) ± standard error (S.E) with the same letter in each column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$. *** $P \leq 0.001$.

In Korarima, root number and root length were decreased with increasing Imazalil concentration (0-4 mg/l) in devoid of cytokinins [17]. According to Werbrouck and Debergh [7], in *S. floribundum* when Imazalil (4 or 16 mg/l) was applied without BA, root number and root length were reduced, but no new shoots were developed. In this study, thus, 2.5-10 mg/l Imazalil even though promoted root elongation of *S. raeseri* shoot tip explants, however led to a simultaneous decrease in root number and rooting percentage. In particular, rooting (66.67%) and callus formation (100%) were highest in the control and in explants treated with 1 mg/l Imazalil. In addition, imazalil (2.5-10 mg/l) decreased callogenesis percentage of *S. raeseri* explants (Table 2). Similarly, in Gerbera '*Rosabella*' (bushy) cultivar, Imazalil reduced callus formation [20].

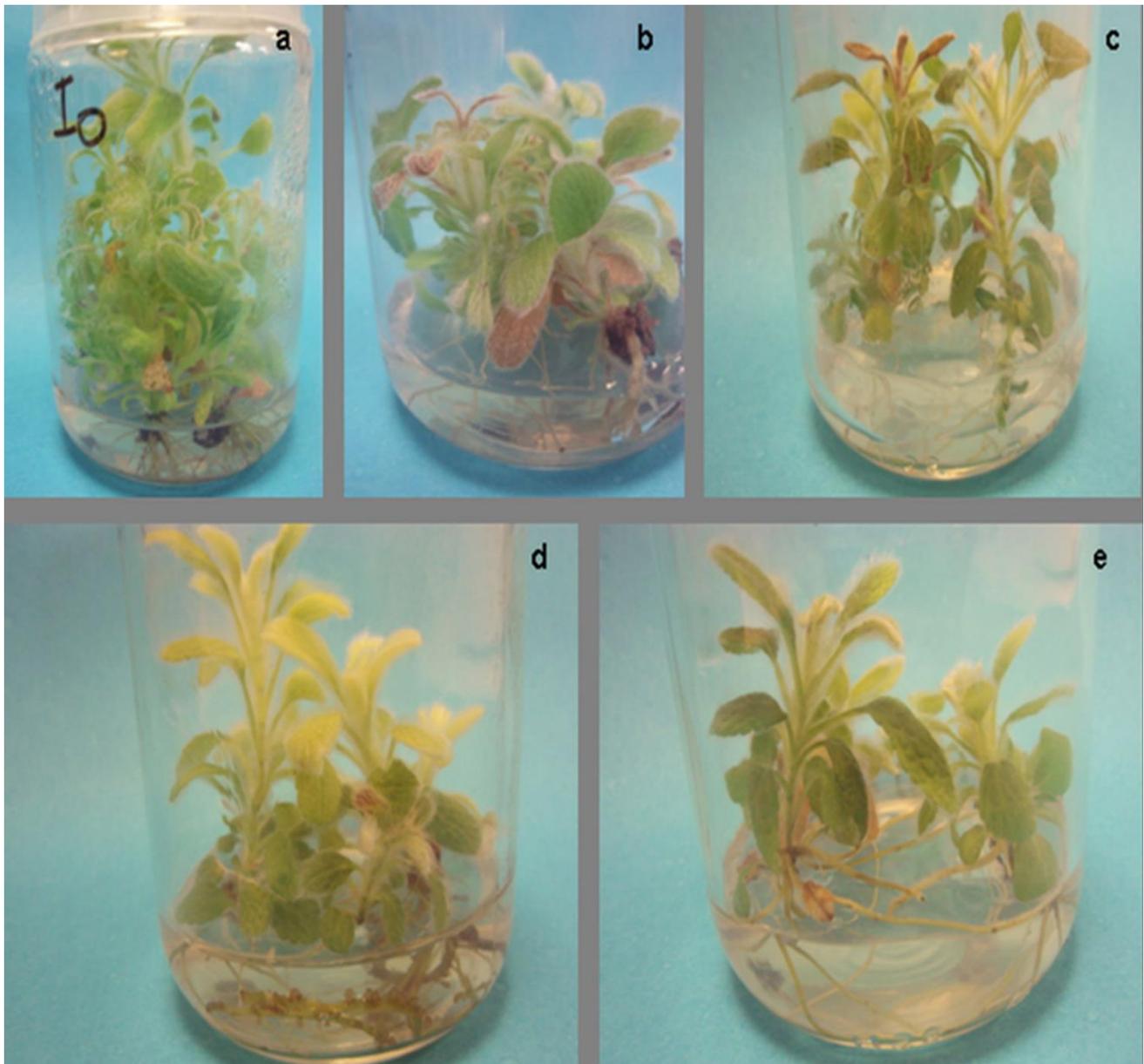


Fig. 1. Effect Imazalil concentration combined with 0.5 mg/l KN + 0.05 mg/l NAA on micropropagation of *S. raeseri* Boiss & Heldr. subsp. *raeseri*: **(a)** Control (Imazalil-free), **(b)** 1 mg/l, **(c)** 2.5 mg/l, **(d)** 5 mg/l, **(e)** 10 mg/l Imazalil.

In the current study, 95% *ex vitro* survival percentage of *S. raeseri in vitro* rooted explants originated from Imazalil experiment was recorded (Fig. 2).



Fig. 2. *Ex vitro* acclimatization and hardening of rooted microcuttings derived from Imazalil-enriched and Imazalil-free culture media in the mist system under unheated greenhouse conditions.

Experiment 2: Effect of Carbendazim on micropropagation of *S. raeseri*

The combined effect 0.5 mg/l BA + 1 mg/l Carbendazim gave the best results with respect to the number of shoots/ explant (5.77), shoot length (16.1 mm) and shoot multiplication percentage (100%) (Fig. 3b) compared to the control (0.5 mg/l BA, Carbendazim-free) (Table 3 and Fig. 3a). Carbendazim applied at 1 mg/l in combination with 0.5 mg/l BA promoted *in vitro* shoot proliferation of *S. raeseri* explants in relation to BA alone, thus, Carbendazim showed cytokinin-like activity by enhancing the stimulatory effect of BA on shoot proliferation. In accordance to our findings in *S. raeseri*, the positive effect of bavistin (100-300 mg/l) on *in vitro* shoot regeneration has already been reported in several other plant species including *Solanum viarum* Dunal (Tropical Soda apple) [22], *Centella asiatica* (L.) Urban [23], *Asclepias curassavica* (L.) (Tropical milkweed) [24], peppermint (*Mentha piperita* L.) [25], *Stevia rebaudiana* [26] and Nepali's Kutki (*Picrorhiza scrophulariiflora* Pannell.) [27].

Table 3. Effect of Carbendazim concentration (0-400 mg/l) combined with 0.5 mg/l BA on shoot number/ explant, shoot length (mm), shoot multiplication percentage (%), callus formation and vitrification percentages (%) in *S. raeseri* Boiss & Heldr. subsp. *raeseri*.

Carbendazim (mg/l)	Shoot number /explant	Shoot length (mm)	Shoot multiplication percentage (%)	Callus formation percentage (%)	Vitrification percentage (%)
Control	4.64 ± 0.42 cde	12.17 ± 0.79 ab	90.91 d	100 f	18.18 d
1	5.77 ± 0.74 e	16.10 ± 0.72 c	100 g	100 f	0 a
2.5	2.69 ± 0.28 ab	11.15 ± 0.69 ab	76.92 bc	92.31 e	76.92 g
5	2.86 ± 0.32 ab	13.66 ± 0.84 bc	92.86 ef	78.57 c	14.29 c
10	3.93 ± 0.70 bcd	11.48 ± 0.83 ab	86.67 e	66.67 b	33.33 e

25	2.92 ± 0.56 ab	16.10 ± 1.28 c	75.00 b	91.67 d	8.33 a
50	2.07 ± 0.25 a	12.05 ± 0.91 ab	64.29 a	57.14 a	42.86 f
100	5.23 ± 0.65 cde	9.67 ± 0.62 a	92.31 ef	61.54 ab	23.08 d
200	3.82 ± 0.60 bc	13.60 ± 0.73 bc	81.82 bcd	54.55 a	0 a
400	5.57 ± 0.62 de	13.01 ± 1.10 b	81.82bcd	92.86 e	0 a
P-values	0.000***	0.000***	0.000***	0.000***	0.000***

Means (n=15) ± standard error (S.E) with the same letter in each column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$. *** $P \leq 0.001$.

In the experiment with *S. raeseri*, 1 mg/l Carbendazim + 0.5 mg/l BA resulted in 100% shoot proliferation (Table 3). According to Baskaran and Jayabalan [28], TDZ, BA, bavistin or Carbendazim and NAA combinations positively influenced adventitious shoot regeneration of *Psoralea corylifolia* L. (Fabaceae). In *S. raeseri*, however, there was a decline in shoot multiplication percentage in the 10-50 mg/l Carbendazim concentration range (Table 3). In *S. rebaudiana in vitro* culture, 200 mg/l bavistin + 2 mg/l BA decreased the frequency of axillary bud explants for regeneration when compared with the cultures containing only bavistin [26]. Regarding *S. raeseri* explants, 1-400 mg/l Carbendazim neither promoted nor inhibited shoot elongation (Table 3). On the other hand, in *Cordyline terminalis* and *Prunus avium* species, Carbendazim concentrations above 160 mg/l had a negative impact on the height of seedlings [29], while in the Indian medicinal herb *B. monniera*, 300 mg/l Carbendazim contributed to a remarkable elongation of the explants [30].

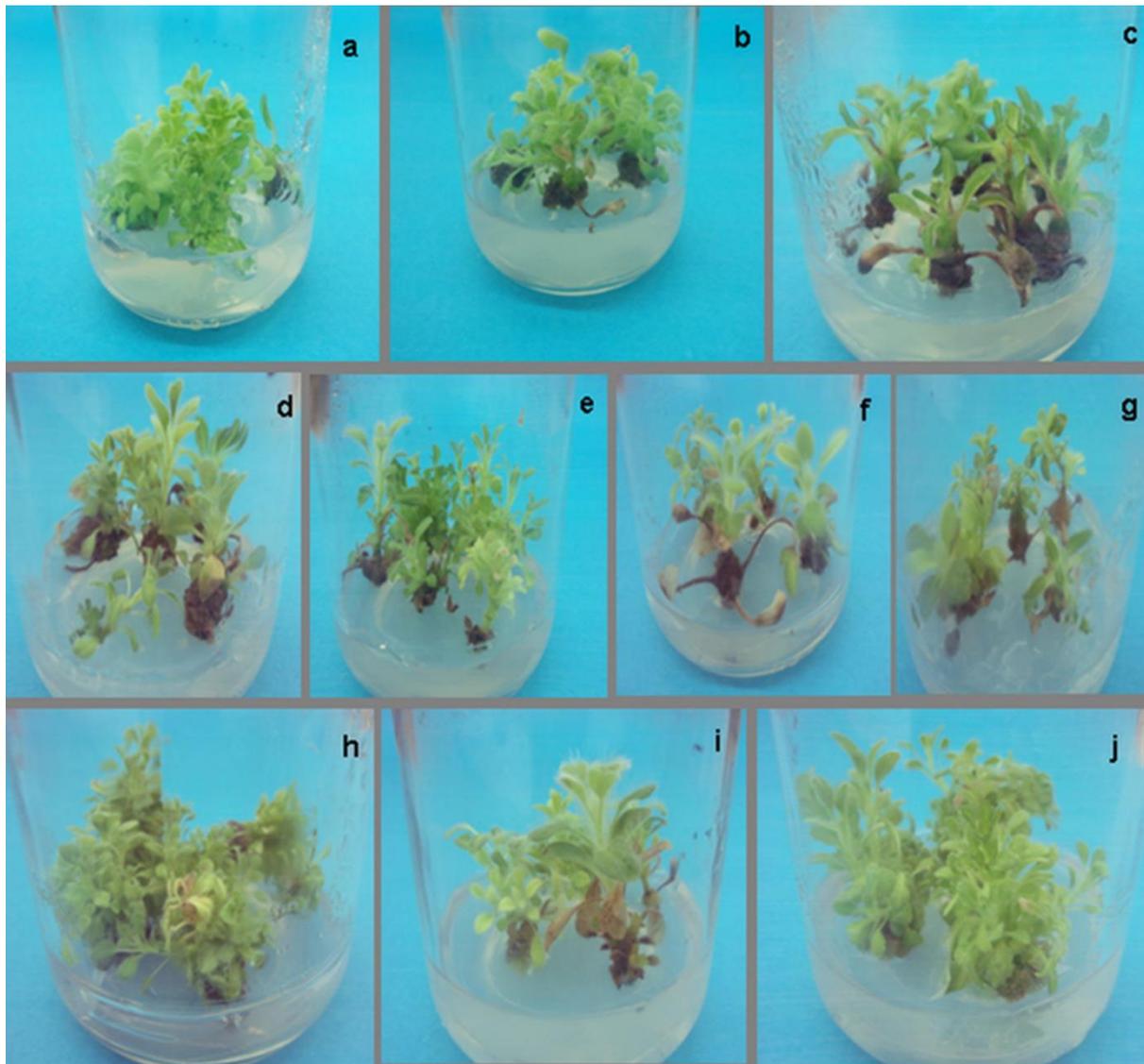


Fig. 3. Effect of Carbendazim concentration (0-400 mg/l) combined with 0.5 mg/l BA on *in vitro* shoot proliferation of *S. raeseri* Boiss & Heldr. subsp. *raeseri*: **(a)** Control (Carbendazim-free), **(b)** 1 mg/l, **(c)** 2.5 mg/l, **(d)** 5 mg/l, **(e)** 10 mg/l, **(f)** 25 mg/l, **(g)** 50 mg/l, **(h)** 100 mg/l, **(i)** 200 mg/l, **(j)** 400 mg/l Carbendazim.

In this research study, *S. raeseri* shoot tip explants treated with 2.5-400 mg/l Carbendazim exhibited decreased callus induction percentages. Callogenesis was 100% in the control treatment, and with 1 mg/l Carbendazim (Table 3). According to Nandi et al. [31], in *Taxus baccata* ornamental plants, Carbendazim induced callogenesis (>90%). In this experiment with *S. raeseri*, hyperhydricity symptoms were observed both in the control and in 2.5-100 mg/l Carbendazim treatments. Furthermore, no hyperhydricity symptoms were evident when explants were treated with 200 or 400 mg/l Carbendazim (Fig. 3i-3j). Instead, the vitrification problem was evident and more extensive in the case of the control (18.18%) and less severe with 2.5-100 mg/l Carbendazim (8.33-76.92%) (Fig. 3c-3h). However, there were no signs of vitrification in *S. raeseri* microshootlets treated with 1 mg/l or 200-400 mg/l Carbendazim (Table 3). In Nepali's Kutki microplants, no vitrification was observed with 50-100 mg/l bavistin, nevertheless, vitrification was increased about 49.2% when 400 mg/l bavistin were used [27]. Carbendazim did not affect shoot proliferation of *S. raeseri* explants adversely and did not lead to the appearance of necrotic symptoms.

Conclusions

From the results obtained it is evident that the imidazole fungicide Imazalil cannot be used in tissue culture systems as a promoting shoot proliferation agent, however, its use is recommended only for enhancing root elongation of *S. raeseri* shoot tip explants. The use of the benzimidazole fungicide Carbendazim may be useful as a media supplement to develop efficient protocols as it eliminated the contaminating microorganisms and subsequently favoured *in vitro* shoot regeneration of *S. raeseri* explants. Because of unrestricted exploitation of this useful medicinal plant, its population is fast receding from the natural habitat, therefore, effective conservation methods are required for its protection from extinction. This protocol can be successfully used for clonal micropropagation of *S. raeseri* Boiss & Heldr. subsp. *raeseri* in a commercial scale at short time rather than natural conditions and in genetic transformations. The study is useful in establishing technological improvement in *S. raeseri* by genetic engineering and transferring new improved quantitative and qualitative traits in several mountain tea species of *Sideritis* genus.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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