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Clonal propagated 'Ek Pothi Lehsun' as a potential antifungal agent against *Candida* sp.

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ABSTRACT

'Ek Pothi Lehsun', also known as snow mountain garlic, is a type of garlic grown in the high mountainous region of Jammu and Kashmir state of India. The present study aimed to develop a protocol for propagating snow mountain garlic *in-vitro* using corm seed as an explant. The study also assessed the antifungal potential of in vitro-grown bulbils against different *Candida* species. Four different concentrations of NAA and 2,4-D were tested for their effectiveness in promoting root formation, and eighteen different combinations of BAP (µM), KN (µM) and TDZ (µM) were investigated for effective proliferation of shoots with varied lengths. Shoot with maximum length (5.03±1.40) was obtained in MS medium containing 1.0 µM TDZ after 24 days of inoculation, whereas MS basal media was found effective for rooting plantlets. Rooted micro shoots were acclimatized successfully in hardening treys with a percent survival of nearly 80%. Seven different concentrations of Sucrose, i.e. 5%, 7%, 10%, 15%, 17%, 20%, and 25% were investigated for effective bulbil formation. Bulbil with a maximum diameter of 0.86 cm was obtained in 20% sucrose-containing MS media in 5 days. Further, the antifungal potential of aqueous extract (TC-SMG) of *in vitro* grown bulbils was investigated against three *Candida* sp. A zone of inhibition of 22.30±0.33 mm, 17.3±0.33 mm and 19.3±0.33 mm was observed against *C. albicans*, *C. tropicalis* and *C. glabrata* respectively, by using 200 mg/mL extract after 24 hrs depicting the remarkable potential of TC-SMG as an antifungal agent. *In vitro* culture of snow mountain garlic has demonstrated promising antifungal properties against *Candida* species.

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1 Introduction

For millennia, plants have served humanity as sources of beneficial drugs, food, flavouring agents, colourants, binders and lubricants. They have been used to develop and maintain various communities and cultures' physical, psychological and spiritual health (Davis and Choicy 2024). *Allium* is one such plant genera with many valuable medicinal properties. Several health benefits of this plant species have already been documented in Charaka Samhita, one of the oldest Indian medicinal treatizes (Devi et al. 2014). *Allium* has been recognized as one of the initial instances of the plant to be utilized for medicinal purposes. This genus includes many economically important crops like garlic, onion, and other ornamental species (Devi et al. 2014). *Allium sativum* (garlic) is a valuable bulbous crop of this genus, widely used as a spice/ condiment throughout India. It is a natural antibiotic and a remedy for various physical ailments (Parekh and Chanda 2007; Papu et al. 2014). Earlier, various civilizations, viz., Egyptian, Phoenicians, Greek, Indian, Roman, Babylonian, and Chinese, have demonstrated this plant species to be used for curing many ailments such as heart disorders, arthritis, pulmonary disorders, uterine growths, skin disease, symptoms of ageing, diarrhoea, headache, worms and tumours. Egyptians have been demonstrated to provide garlic to the labour force involved in heavy pyramid construction work (Woodward 1996; Sasi et al. 2021).

'Ek pothi lehsun', commonly known as snow mountain garlic or 'Kashmiri garlic', is a type of garlic found to grow in mountainous regions of the Himalayas at an altitude of 1800 meters from MSL. Solid cloves of snow mountain garlic are the cold, hardy corm seeds developed from the elephant garlic, planted in September or October and harvested in the summers. If left in the field, each clove miraculously transforms into complete elephant garlic in the following year. Earlier, mountaineers were found to use it to enhance their energy levels and remove toxins in extreme environmental conditions (https://specialtyproduce.com/produce/Kashmiri_Garlic_13356.php).

Candida sp. is an opportunistic fungal pathogen of the human oral-gastrointestinal tract. These pathogenic species have been recognized as one of the most prevalent reasons for nosocomial infections in patients (Lemar et al. 2002). Therefore, it is also known as "the disease of the diseased" (Al-Dorzi et al. 2020). Antifungal agents such as flucytosine and amphotericin B (AmB) are conventionally used to treat such infections (Kim et al. 2012). However, with the increase in fungal resistance to conventional medicines and the side effects of using these medicines, especially in immune-compromised patients, there is an urgent necessity to hunt for new sources of alternative medicines. Various studies have indicated the hidden power of garlic (*A. sativum*) as an alternative source of antimicrobial properties (Bayan et al. 2020). It has been found to contain a compound named 'allicin' containing diallylsulphide and thiosulfinate, which has been responsible for its antimicrobial potential (Rounds et al. 2012; Heshmati et al. 2010). Elephant garlic extracts, like other *Allium* species, have also been found to contain eight different thiosulfinates responsible for their antimicrobial activity (Huang and Ren 2013). Snow mountain garlic is a variant species of *Allium*. It has been considered a survival strategy for elephant garlic in various abiotic and biotic stress conditions. Therefore, it might be reasonable to systematically study the effectiveness of snow mountain garlic against various *Candida* sp.

However, the long life cycle and low productivity have made this plant species less acceptable among the farmers. Further, the necessity of environmental stress conditions has restricted this crop plant to the farmers of particular habitats. Therefore, in the present study, an attempt has been made to establish an *in vitro* propagation protocol of this plant species to reduce its life cycle so that increased commercial demand can be satisfied without the requirement of environmental stress conditions followed by an assessment of the effectiveness of water extract of *in vitro* grown bulb (TC-SMG) against different *Candida* species.

2 Materials and Methods

2.1 Collection of plant material and sterilization

Certified snow mountain garlic corm seeds were obtained from Srinagar, Jammu & Kashmir, India. Collected seeds were kept in running tap water for three hours followed by surface sterilization in three steps for establishment of *in vitro* tissue culture of snow mountain garlic which involved sterilization of corm seeds with ethanol (70% (v/v)) for 120 seconds, mercuric chloride (0.04% (w/v, 60 seconds) followed by extensive washing for 4–5 times, with autoclaved distilled water in order to remove the remaining traces of sterilizing agents and then transferred to MS medium having 3% sucrose as carbon source (Morales et al. 2006).

2.2 Establishment of aseptic culture of snow mountain garlic using corm bud as explant

In-vitro culture of snow mountain garlic was established using corm seeds containing axillary bud as a source of explants (Figure 1). For this, the thick coat of each of these seeds was removed using a scalpel blade to expose the bud. After this, seeds were shifted to MS medium augmented with 3.00% (w/v) sucrose, 0.78% agar, and numeral concentrations of plant growth regulators, i.e., 6-benzylaminopurine (BAP), thidiazuron (TDZ) and kinetin (KN) for effective shoot multiplication. Agar-agar was added to the culture medium after setting the pH (5.80). The medium thus prepared was transferred to various culture vessels for effective sterilization in an autoclave at a pressure of 15 psi for 15 min. A Laminar air chamber was used to inoculate explants on a sterilized medium under aseptic conditions. All the inoculated cultures were then kept at a

Figure 1 A) Cloves of Snow Mountain Garlic B) Peeled cloves exposing the axillary bud

temperature of 22 ±2°C, humidity of 70–80%, and in photoperiod of 16/8 h light/dark in the culture room containing optimal light intensity (40 µmolm^{-2}s^{-1}) using white fluorescent lamps (cool). Data was recorded 25 days after inoculation.

2.3 Effect of different auxins on root induction from explant

Four different concentrations of NAA (1, 1.5, 3.0, 4.0 µM µM) and 2,4 D (1, 1.5, 3.0, 4.0 µM) were investigated for effective root formation. For this, shoots in the early stages of development were transferred to MS medium containing different concentrations of NAA and 2,4 D.

2.4 Effect of different concentrations of Sucrose on bulb formation

Seven different Sucrose concentrations, 5%, 7%, 10%, 15%, 17%, 20%, and 25%, were studied for effective bulb formation, keeping the standard 3% sucrose concentration as control.

2.5 Hardening and acclimatization of *in vitro* grown plants

Hardening of *in vitro* grown plants of snow Mountain Garlic was carried out in hardening trays. For this, *in vitro* grown plants of 80 days were taken out from tubes and inoculated into hardening media consisting of a mixture of cocopeat and vermicompost in the ratio of 1:1. Before transferring, the plants were given treatment of a fungicide bavistin (0.04%), followed by thorough washing to remove traces of bavistin. Plants were kept in a hardening chamber. Frequent water sprays were given to maintain humidity.

2.6 Preparation of aqueous extract of in vitro grown garlic bulbils

Aqueous extract of in vitro developed garlic bulbils was prepared using the method given by Suleria et al. (2012). Seed bulbs from *in vitro* grown plants were collected and weighed (5.0 g), followed by their thorough homogenization with double distilled water to obtain fine garlic juice. The homogenized mixture was filtered through 2-3 layers of muslin cloth. The resultant aqueous garlic extract was passed through Whatman™ grade 1 filter paper. The recovered filtrate was freeze-dried and stored at 4°C until further use. Different concentrations of garlic extract were prepared by diluting it with sterile water (Suleria et al. 2012). Prior to each antifungal assay, aqueous extract was filtered through a Whatman™ 0.22µm PVDF membrane filter.

2.7 Analysis of the antifungal activity of aqueous extract of Snow Mountain Garlic against different *Candida* species

Antifungal potential of aqueous extract of in vitro grown snow mountain garlic was assessed through agar well diffusion assay. Three species of *Candida* viz., *Candida glabrata*, *C. tropicalis*, and *C. albicans* were obtained from MTCC (Microbial type culture collection), CSIR- IMTECH, Chandigarh, India. Procured fungal cells were grown on Yeast Malt Agar (YMA) at 30 °C and sub-cultured 2-3 times to confirm the purity and viability of cells. Single colony of each species was activated in YM broth for 4-5 hrs (OD was 0.4- 0.5). Each activated pathogen inoculum was spread on YM agar containing petridishes using a sterile L-shaped spreader and allowed to dry at room temperature for 2-3 minutes. Wells of 6mm diameter were made by using a cork borer. Six different concentrations (200, 100, 80, 60, 40, 20 mg/mL) of garlic extract were dispensed in the holes, followed by incubation at 30 °C for 24 and 48 hrs, respectively. The negative control well was poured with sterile MQ water. The zone of inhibition diameter was measured with an antibiotic zone scale after 24 and 48 hrs for each selected pathogenic strain, respectively.

2.8 Minimum Inhibitory Concentration (MIC)

The MIC value of TC-SMG (aqueous extract) was analyzed using the Tholen et al. (2004) protocol with slight modifications. This study used six different concentrations viz., 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 mg/mL of in vitro grown tissue culture (TC-SMG extract were prepared by serial dilution). Twenty microliters of each concentration was dispersed in 80 µL of YM broth, followed by

100 µL of cell suspension inoculums with 0.003-0.004 OD at 600 nm, which was added to the 96-well plate. Two wells of 100 µL of YM broth and fungal cell suspension were kept as a positive control. Plates were incubated at 30 °C, and OD was measured at 600 nm at 24 and 48 hours using an ELISA microplate reader (Thermofischer, USA).

2.9 Statistical analysis

All the experimental results were presented as the mean ± standard error (SE), and all experiments were performed in triplicate. Independent t-test was used in SPSS 17.0 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) to determine the significance of the results. A probability (p) value of ≤ 0.05 was treated significantly for ANOVA and the marked correlations between the numeral assays.

3 Results

Sterilized cloves of snow mountain garlic were transferred to MS medium supplemented with different combinations of auxins and cytokinins. Germination started within 10-12 days in explants of snow mountain garlic.

3.1 Effect of different cytokinins on shoot induction from explant

Hard coat of sterilized cloves of Kashmiri garlic was removed carefully and transferred to MS medium containing distinct concentrations of BAP (µM), KN (µM), and TDZ (µM). A total of 18 various compositions of three selected cytokinins were studied to assess their effect on shoot proliferation of varied lengths. MS basal without the addition of any growth hormones was used as a control. Single shoot was found to develop in all the investigated hormones. In the case of BAP and KN, the highest length of shoot, i.e., 1.36±0.23 cm and 1.43±0.133 cm, was witnessed in a medium containing 5.0 µM concentration of each respective hormone. However, the maximum length of the shoot (5.03±1.40) was observed in MS medium supplemented with 1.0 µM TDZ by direct organogenesis after 24 days of inoculation, whereas no shoot formation was observed in control. A sudden increase in shoot length was observed in shoots after 15 days of inoculation, which showed further enhancement in length with time. Thus, basal medium (MS) containing 1.0 µM TDZ was selected as the optimum medium for effective shoot proliferation compared to BAP and KN supplemented medium (Table 1).

Table 1 Effect of different concentrations of cytokinins on shoot development

S. N.	Medium Code	Medium composition	No of Shoots	Length of shoots
1.	SMGK0A	MSBM	0.00^{a}	0.00^{a}
2.	SMGK1	MSBM + 1.0 µM KN	0.67 ± 0.33^{c}	0.50 ± 0.05^{ab}
3.	SMGK2	MSBM + 2.0 µM KN	1.00 ± 0.00^{d}	1.43 ± 0.14^{bc}
4.	SMGK3	MSBM + 3.0 µM KN	1.00 ± 0.00^{d}	1.00 ± 0.15^{b}
5.	SMGK5	MSBM + 5.0 µM KN	1.00 ± 0.00^{d}	1.43 ± 0.13^{bc}
6.	SMGK7	MSBM + 7.0 µM KN	0.67 ± 0.33^{c}	0.80 ± 0.44^{ab}
7.	SMGB1	MSBM + 1.0 µM BAP	1.00 ± 0.00^{d}	1.00 ± 0.19^{b}
8.	SMGB3	MSBM + 3.0 µM BAP	1.00 ± 0.00^{d}	1.03 ± 0.08^{b}
9.	SMGB5	MSBM + 5.0 µM BAP	0.67 ± 0.33^{c}	1.36 ± 0.23^{bc}
10.	SMGB7	MSBM + 7.0 µM BAP	0.33 ± 0.33^{b}	0.32 ± 0.33^{a}
11.	SMGT0.5	MSBM + 0.5 µM TDZ	1.00 ± 0.0^{d}	3.77 ± 0.89^{d}
12.	SMGT1	MSBM + 1.0 µM TDZ	1.00 ± 0.0^{d}	5.03 ± 1.40^{e}
13.	SMGT3	MSBM + 3.0 µM TDZ	1.00 ± 0.0^{d}	4.87 ± 0.73^{e}
14.	SMGT5	MSBM + 5.0 µM TDZ	1.00 ± 0.0^{d}	2.30 ± 0.05^{c}
15.	SMGG0.5	MSBM + 5.0 µM TDZ+ 0.5 µM GA3	1.00 ± 0.0^{d}	2.06 ± 0.08^{c}
16.	SMGG1	MSBM + 1.0 µM TDZ+ 1.0 µM GA3	1.00 ± 0.0^{d}	3.17 ± 0.07^{d}
17.	SMGG3	MSBM + 3.0 µM TDZ+ 1.0 µM GA3	1.00 ± 0.0^{d}	3.20 ± 0.52^{d}
18.	SMGG5	MSBM + 5.0 µM TDZ+ 1.0 µM GA3	1.00 ± 0.0^{d}	2.63 ± 0.06^{cd}

MSBM - MS basal medium; Values are in means ± SEM of three determinations; Values with distinctive superscript (little letter set) letters inside a column were altogether distinctive ($p\leq0.05$)

Table 2 Effect of different concentrations of auxins on root development

S. N.	Medium Code	Concentration of NAA (µM)	No of roots (cm)	Length of roots (cm)
1.	SMG0	0.0 µM NAA	4.33±0.33[a]	1.43±0.14[a]
2.	SMG1	1.0 µM NAA	4.00±0.57[a]	1.50±0.11[a]
3.	SMG2	1.5 µM NAA	2.33±0.33[b]	1.27±0.03[ab]
4.	SMG3	3.0 µM NAA	1.33±0.33[c]	0.91±0.02[b]
5.	SMG4	4.0 µM NAA	0.67±0.33[d]	0.58±0.11[c]

Values are means ± SEM of three determinations. The values having distinctive superscript (little letter set) letters inside a column were altogether distinctive ($p \leq 0.05$).

3.2 Effect of different auxins on root and callus induction from explants

Four different concentrations of NAA **(**µM) were studied for effective root formation. Initiation of root development was observed after 10 days of inoculation. A significant variation in the number of roots was observed in a medium supplemented with different concentrations of NAA **(**µM). Among all the studied concentrations of NAA, the highest no of roots (4.0±0.578) with the highest length (1.5±0.11 cm) were found to develop in 1.0 µM concentration of NAA. However, the maximum no of roots (4.33±0.33) was found to develop in the MS basal medium (control) (Table 2).

In the case of 2,4 D, callus formation was observed in all the studied concentrations of 2,4D viz. 1 µM, 1.5 µM, 3 µM and 4 µM (Figure 2).

Based on the above results, MS medium supplemented with 1.0 µM TDZ was selected as the optimum medium for effective shoot and root development and selected further for effective multiplication of plants (Figure 3).

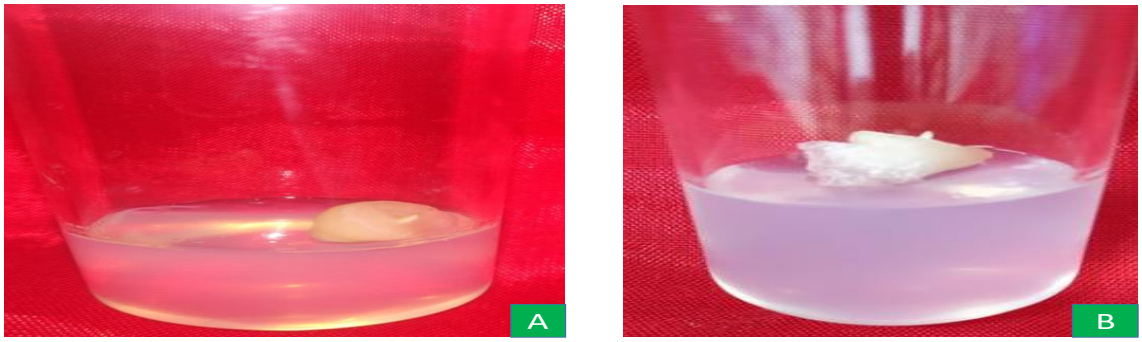

Figure 2 A) Inoculation of explant on MS medium containing 2, 4 D B) Formation of callus (15 days)

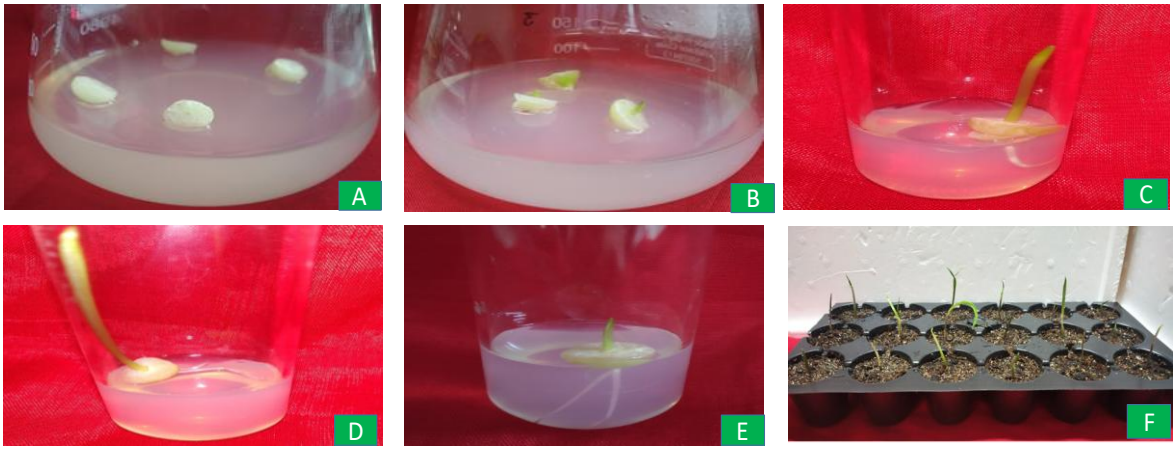

Figure 3 A) Explant transferred to MS medium supplemented with different concentrations of growth hormones B) Initiation of germination (10-12 days) C) Initiation of shoot formation on MS medium supplemented with 1.0 µM TDZ D) Elongation of shoot (15 days) E) Development of roots in MS basal medium F) Hardening of plants in controlled conditions

Table 3 Effect of various sucrose concentrations on bulb formation

S. N.			
1.	3.0	25.33±0.88^{d}	0.27±0.008^{a}
2.	5.0	14.67±0.67^{c}	0.28±0.011^{a}
3.	7.0	14.00±0.58^{c}	0.49±0.052^{b}
4.	10.0	12.33±0.33^{b}	0.55±0.014^{b}
5.	15.0	5.00±0.58^{a}	0.74±0.015^{c}
6.	17.0	4.67±0.33^{a}	0.84±0.012^{d}
7.	20.0	5.00±0.00^{a}	0.89±0.012^{d}
8.	25.0	5.00±0.00^{a}	0.88±0.008^{d}

Values are as means ± SEM of three determinations. Values with distinctive superscript (little letter set) letters inside a column were altogether distinctive ($p \leq 0.05$).

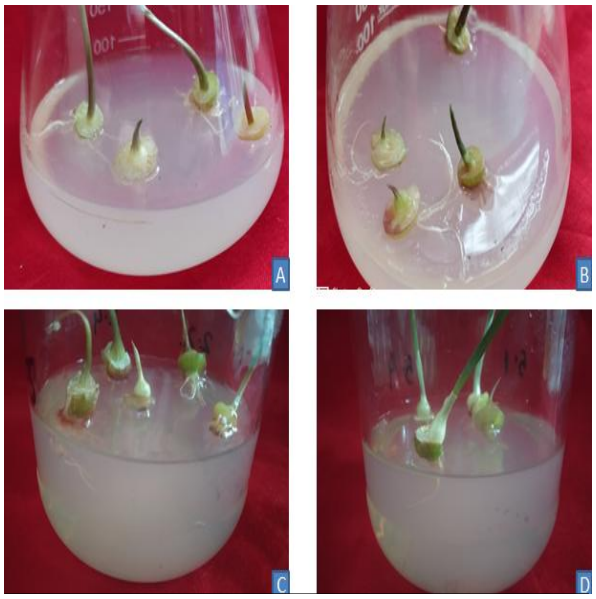

Figure 4A: A) Explant in 3% sucrose B) Explant in 5% sucrose C) Explant in 7% sucrose D) Explant in 9% sucrose

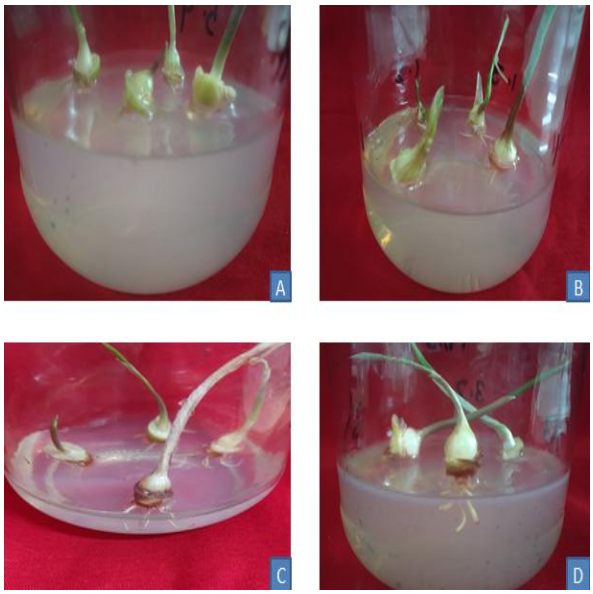

Figure 4B: A) Explant in 15% sucrose B) Explant in 17% sucrose C) Explant in 19% sucrose D) Explant in 20% sucrose

3.3 Effect of different concentrations of Sucrose on bulb formation

Development of corms from shoots can play an important role in their successful acclimatization and *in vitro* mass cultivation. Corms formed under invitro conditions are better adapted to storage conditions. Thus, in this study, six different concentrations of Sucrose in MS medium, i.e., 5, 7, 10, 15, 17, 20, and 25%, were investigated for *in vitro* corm development while 3% sucrose concentration was used as control. The average diameter of bulb was noticed to enlarge with the increment in concentration of Sucrose, whereas the duration of bulb development was found to decrease with the increase of sucrose concentration. A maximum diameter of 0.86 cm was obtained in MS medium supplemented with 20% sucrose in 5 days, while further increase in the concentration of Sucrose has left no further impact on the increase of diameter as well as on-time duration. Further, a diameter of 0.25 cm was observed in MS medium supplemented with 3.0% sucrose (control) after an average duration of 25.33 days (Table 3, Figure 4A-B). Investigation of liquid medium containing optimized concentration of Sucrose, i.e., 20% for effective bulbil formation, has resulted in a further decrease in time duration (3 days) of bulb formation (Figure 5).

3.4 Hardening and acclimatization of *in vitro* grown plants

Rooted plants were hardened and acclimatized in plastic trays in a hardening chamber. A frequent sprinkling of water was carried out to ensure humidity maintenance. Approximately 80% survival of plantlets was observed after 3 weeks of transplantation (Figure 3).

Figure 5 Development of bulbil in liquid MS media supplemented with 20% sucrose (3 days)

3.5 Analysis of the antifungal activity of aqueous extract of Snow Mountain Garlic against different *Candida* species

The agar well diffusion method evaluated the effectiveness of TC-SMG aqueous extract against 3 selected *Candida* species. Six different concentrations of TC-SMG viz., 20, 40, 60, 80, 100, and 200 mg/mL were tested against all the selected pathogens at 24 and 48 hrs, respectively. In the case of *C. albicans*, the maximum zone of inhibition (22.30±0.33 mm) was observed using 200 mg/ml extract after 24 hrs. Further, a non-significant inhibition zone reduction was observed after 48 hrs (Table 4). In the case of *C. tropicalis*, no inhibition was observed by using 20 mg/mL concentration at both time intervals (Table 4). However, the zone of inhibition was found to increase by further increasing the concentration, whereas the maximum zone of inhibition was achieved by using 200 mg/mL of TC-SMG after 24 (17.3±0.33 mm) and 48 hrs (16.3±0.88 mm). Similarly, in the case of *C. glabrata*, no zone of inhibition was observed using 20 mg/mL TC-SMG aqueous extract, and the highest zone of inhibition (19.30±0.33 mm) was reported after 24 hrs (Table 4). In all three

Table 4 Antifungal activity of various concentrations of TC-SMG extracts against selected *Candida sps*

S. N.	TC-SMG Concentration (mg/mL)	Zone of Inhibition (mm) at 24 hours			Zone of Inhibition (mm) at 48 hours			Positive control
		C. albicans	*C. tropicalis*	*C. glabrata*	*C. albicans*	*C. tropicalis*	*C. glabrata*	
1.	20	13.70±0.33^{a}	0.00±0.00^{a}	0.00±0.00^{a}	7.00±3.51^{a}	0.00±0.00^{a}	0.00±0.00^{a}	Growth
2.	40	16.70±0.33^{b}	12.00±0.00^{b}	4.00±4.00^{b}	14.70±0.33^{b}	6.70±3.33^{b}	4.00±4.00^{a}	Growth
3.	60	18.70±0.33^{c}	14.30±0.33^{c}	8.30±4.26^{c}	16.70±0.33^{bc}	12.30±0.33^{c}	7.70±3.93^{b}	Growth
4.	80	19.70±0.33^{c}	15.70±0.33^{d}	15.00±0.58^{d}	17.70±0.33^{bc}	14.30±0.33^{d}	13.30±1.33^{c}	Growth
5.	100	20.70±0.33^{d}	16.30±0.33^{d}	16.70±0.67^{de}	18.70±0.33^{c}	14.70±0.33^{d}	15.00±1.00^{d}	Growth
6.	200	22.30±0.33^{e}	17.30±0.33^{e}	19.30±0.33^{e}	21.00±0.58^{d}	16.30±0.88^{e}	18.70±0.67^{e}	Growth

Values are as means ± SEM of three determinations. Values with distinctive superscript (little letter set) letters inside a column were altogether distinctive ($p \leq 0.05$).

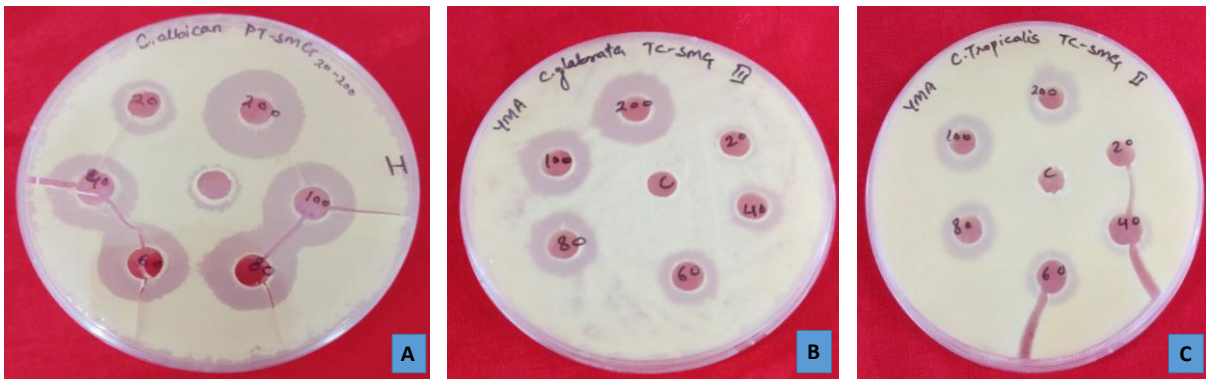

Figure 6 Antifungal effect of different concentrations of TC-SMG after 24 hrs on A) *Candida albicans*, B) *C. glaborata*, C) *C. tropicalis*

cases, depicting its remarkable potential to be used as an antifungal agent, a non-significant reduction in the size inhibition zone was observed after 48 hrs (Figure 6).

3.6 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) against all the test pathogens was also evaluated in the range of 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 mg/ml. A MIC_{99} value of 3.2 mg/mL was observed for *C. albicans* at 24 and 48 hrs. For *C. tropicalis, a* MIC_{99} value of 1.6mg/mL was observed at 24 hrs, whereas at 48 hrs, it was observed to be 3.2 mg/mL. Further, in the case of *C. glabrata*, MIC_{99} of 6.4 mg/mL was obtained at both 24 and 48 hrs. From these results, it is visible that *C. tropicalis* has more susceptibility towards TC- SMG aqueous extract than the other two pathogens. On the other hand, *C. glabrata* was found more resistant to TC-SMG extract as it has shown the highest MIC amongst all three *Candida* spp.

4 Discussion

For thousands of years, garlic has remained an important herb for human consumption. When antibiotics and other pharmaceutical products did not exist, a bulb of garlic was used to represent the pharmaceutical industry due to its broad spectrum effects (Petrovska and Cekovska 2010). Various clinical investigations have revealed many beneficial effects of garlic, such as (i) reduced risk factors towards cardiovascular disorders, (ii) cancer risk, (iii) enhanced antioxidant and antimicrobial activity, and (v) detoxification of foreign components and liver protection (Aviello et al. 2009; Colín-González et al. 2012). These therapeutical properties of garlic and other members of the genera *Allium* have been attributed to various organosulfur compounds, and the most important sulfurous compound present in intact bulbs is allicin (S-allylcysteine sulfoxide). In addition to this, whole bulbs have also been found to contain several other sulphur compounds such as g-glutamyl-S-allylcysteine (GSAC), S-methylcysteine sulfoxide (methiin), S-trans-1-propenylcysteine sulfoxide, S-2-carboxypropylglutathione and S-allylcysteine (SAC), though some of these are available in much smaller amounts (Amagase 2006). These sulphur-rich compounds have been suggested to be responsible for the various medicinal properties of garlic (Iciek et al. 2009).

Snow mountain garlic is one of the variants found to grow in the mountainous region of Jammu & Kashmir state of India. In ancient times, snow mountain garlic has been observed to be used by various mountain climbers to regain energy and by Greeks to increase the efficiency of athletes participating in Olympics (Sasi et al. 2021). In literature, very little information is available regarding snow mountain garlic. However, for the last few years, an increment in commercial demand for snow mountain garlic has been observed. This increased demand in the market can be attributed to its reduced pungent flavor and beneficial medicinal properties, such as anti-inflammatory, antioxidant, and arthritic properties (Kaur et al. 2024). This garlic is a good source of various minerals, viz., manganese, copper, selenium, phosphorus, and vitamins B6 and C (Kaur et al. 2022).

However, the long life cycle, low productivity, and requirement of long, cold winters have restricted this species to specific areas of the country. A single seed bulb can grow into one plant only, so the availability of a large stock of seed corms is another limitation of its large-scale propagation. Thus, *in vitro* propagation and the development of seed bulbs are important approaches to quickly producing many propagules. In the present study, corm seeds containing axillary bud were used as a source of explant and inoculated on MS medium containing eighteen different concentrations of three selected cytokinins viz., BAP (µM), KN, and TDZ (µM) for efficient shoot proliferation, whereas, basal MS medium was used as control. Maximum shoot length was observed in MS medium supplemented with 1.0 µM TDZ, whereas BAP and KN were less efficient for shoot proliferation. Similarly, Mahajan et al. (2013) reported the lower efficiency of the combinations of BAP and KN for in vitro shoot proliferation compared to individual concentration. However, for the first time, the effect of TDZ has been studied in our investigation, and it has been found more promising than BAP and KN for *in vitro* shoot development.

The long life cycle and development of fewer seed propagules are the key limitations of large-scale cultivation of this species. Therefore, an attempt has been made to develop *in vitro* corm seeds that can be distributed to farmers and can be utilized to satisfy increased commercial demands. For this, different concentrations of Sucrose were applied in MS media. An increase in the bulb's diameter was found with the increase in sucrose concentration. This increase in bulb size with the increase in sucrose concentration can be attributed to the enhancement in the starch and total carbohydrate content (Khokhar 2022). Further, a liquid medium containing an optimized concentration of Sucrose was found to develop corm seed using less medium quantity, depicting the cost-effective *in vitro* development of bulb. It can be attributed to the ease of nutrient uptake resulting in bulbs' development. *Candida* sp has been observed to be able to switch between yeast and hyphae forms, ultimately leading to the formation of biofilms and developing resistance to various antifungal agents (Brand 2012). Since ancient times, plants have been renowned as natural sources of medicine. Several plant species viz., *Osmium sanctum* (Khan et al. 2010); *Glycyrrhiza glabra* (Martins et al., 2016); *Euphorbia hirta* (Rajeh et al., 2010); *Terminalia bellerica* (Valsaraj et al. 1997) have been tested against various *Candida* sp. However, none of these plant products has reached the marketing stage due to insufficient information and efficacy. Therefore, hunting for new plant species with antifungal properties is an alternative to solve these problems.

In the present investigation, aqueous extract of *in vitro* grown Snow Mountain garlic bulbs (TC-SMG) were studied for their efficacy against three *Candida* sp. viz., *C. albicans*, *C. glabrata*, and *C. tropicalis* after 24 and 48 hrs by agar well diffusion method. Six different concentrations were studied against all the selected pathogens. The diameter of the zone of inhibition was found to increase with the increase in extract concentration in all three cases at both time durations. Further, a non-significant reduction in the diameter of the zone of inhibition was observed after 48 hrs, depicting the remarkable potential of this extract to be used as an antifungal agent against different *Candida* sp, specifically in *C. albicans* where the zone of inhibition was found in all concentrations. *Candida* cidal activity of snow mountain garlic has also been investigated by Kaur et al. (2022). It has been suggested that allicin, the major compound found in garlic, can inhibit the thiol-containing amino acids and proteins, thus resulting in the interference in cell metabolism and ultimately leading to the death of the organism (Ankri and Mirelman 1999; Soliman et al. 2017, Kaur et al. 2023).

Conclusion

Food commodities with medicinal properties have remained popular for curing many health problems since immemorial and are being continued until today. Snow mountain garlic is one such plant with several medicinal properties but with a long life cycle and limited productivity. In the present investigation, an effective micropropagation protocol for multiplication of this plant followed by the development of bulbils under in vitro conditions for a reduction in its life cycle in order to their usage as seeds have been investigated and found that 1.0 µM TDZ and 20gm Sucrose can be a best suitable combination for the in-vitro micropropagation of this garlic. Further, water extract at the 200 mg/ml concentration was found fully effective in suppressing *Candida* species growth completely. It will be interesting to determine the effect of this extract on the molecular level of *Candida* proteins targeted by the extract. Further, this protocol could be used to commercialize the cultivation of this snow mountain garlic. To our knowledge, this is the first investigation of antifungal properties of aqueous extract of *in vitro* grown bulbs of snow mountain garlic against different *Candida* sp., depicting its strong rationale for industrial applications.

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Author Contributions

AS wrote draft preparation and carried out experiments, SS performed the tissue culture experiments, MKP contributed to the collection of samples, OPC supervised project administration and funding acquisition, and SSa supervised the work and edited the manuscript. All authors have seen the draft copy and approved the final version.

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Competing of Interest

There is no competing interest among the authors.

Ethics approval

There is no need for ethics approval as this investigation was unrelated to any animal or human subject.

Consent for publication

All authors have approved the manuscript and agree with its submission to JEBAS.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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