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ROLE OF BIOLOGICAL HARDENING IN ENHANCING THE SURVIVAL OF *IN VITRO* RAISED POTATO PLANTLETS

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ABSTRACT

The experiment entitled "Role of biological hardening in enhancing the survival of *in vitro* raised potato plantlets" was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, during 2025 to 2026. Biological hardening is an important step in potato micropropagation, as plantlets are highly susceptible to moisture stress and environmental changes during transfer from *in vitro* to *ex vitro* conditions. The study aimed to evaluate the effectiveness of beneficial microbial bioagents, applied individually and in combination with glycerol, in improving the survival and growth of micropropagated potato plantlets of cv. Kufri Himalini. The bioagents used were *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride*, along with 0.5 per cent glycerol as an anti transpirant. Plant survival was recorded during the first, second and third weeks, while plant height, internodal length and root length were assessed initially and after three weeks. Among the treatments, T_{10} (*P. fluorescens*+ *T. viride* + 0.5 % glycerol) performed best, recording 83.33 per cent survival, 19.03 cm plant height, 2.05 cm internodal length and 10.83 cm root length after three weeks. The second-best treatment, T_5 (*P. fluorescens* + *T. viride*), recorded 80.00 per cent survival, 18.17 cm plant height, 2.02 cm internodal length and 10.07 cm root length. The third-best treatment, T_6 (*T. viride* + 0.5% glycerol), recorded 76.67 per cent survival, 17.13 cm plant height, 2.01 cm internodal length and 9.53 cm root length after three weeks. In contrast, the control recorded the lowest survival (56.67 %) and plant height (14.47 cm). The findings indicate that the combined application of *P. fluorescens*, *T. viride* and glycerol is a promising approach for improving the acclimatization, survival and establishment of micropropagated potato plantlets.

KEYWORDS: Biological hardening, potato, micropropagation, *Pseudomonas fluorescens*, *Trichoderma viride*, glycerol, acclimatization.

INTRODUCTION

Potato (*Solanum tuberosum* L.), a natural autotetraploid (2n=4x=48) belonging to the family Solanaceae, is the fourth most important food crop in the world after wheat, rice and maize. It originated in tropical South America and exhibits considerable genetic diversity. Potato is propagated both sexually through true potato seeds and asexually through tubers, with seed tubers being the most widely used planting material. However, conventional tuber-based propagation has several limitations, including low multiplication rates and a high susceptibility to diseases (Mirza *et al*., 2001).

Micropropagation offers an effective biotechnological approach for producing disease-free, genetically uniform potato plantlets through clonal propagation, thereby facilitating the large-scale production of healthy planting material and microtubers. Despite its advantages, micropropagation presents challenges during the transition from *in vitro* to *ex vitro* conditions, as plantlets are highly sensitive to abiotic stresses, such as temperature, light intensity and humidity fluctuations, as well as biotic stresses caused by soil microorganisms. These stresses can result in considerable plantlet losses during acclimatization (Nguyen *et al*., 2016). Therefore, acclimatization is a critical stage that requires the gradual adaptation of *in vitro* plantlets to greenhouse and field conditions. Owing to their poorly developed protective mechanisms and high dependence on humidity, *in vitro* plantlets are susceptible to rapid water loss and wilting. The application of anti-transpirants, such as glycerol, abscisic acid (ABA), paraffin and grease, may help reduce transpiration and improve plantlet survival. Furthermore, pre-hardening strategies, including adjustment of sucrose concentration, application of desiccants and improvement of ventilation, can enhance plantlet establishment under *ex vitro* conditions (El-Din *et al*., 2020).

Biological hardening is a promising strategy for reducing acclimatization stress by introducing beneficial microorganisms into the growing substrate. Since *in vitro*-raised plantlets are generally maintained under aseptic conditions and are physiologically delicate, the introduction of beneficial rhizospheric or endophytic microorganisms, such as *Pseudomonas* and *Bacillus*, can promote root development, plant growth and tolerance to environmental stresses (Mirza *et al*., 2001;

Lugtenburg and Kamilova, 2009). These beneficial bacteria possess several plant growth-promoting traits, including 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, nitrogen fixation, phosphate solubilization and the production of indole-3-acetic acid (IAA), which plays an important role in regulating plant growth and development (Jimtha *et al*., 2014; Zhao *et al*., 2011). In addition, certain bacterial strains exhibit antagonistic activity against plant pathogens, thereby contributing to improved plant health and resilience (Müller *et al*., 2018). Fungal bioagents, particularly *Trichoderma* spp., also serve as effective biocontrol agents and biofertilizers by enhancing root colonization, nutrient uptake, plant growth and productivity (Vinale *et al*., 2008).

In the present study, different combinations of biological hardening agents, with and without the addition of 0.5% glycerol, were evaluated to determine their effects on the survival of in vitro-raised potato plantlets during acclimatization. The study aimed to identify the most effective combination of biological hardening agents and glycerol for improving plantlet survival and successful establishment under ex vitro conditions.

MATERIAL AND METHODS

Experimental Location

The present investigation entitled "Role of biological hardening in enhancing the survival of *in vitro* raised potato plantlets" was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, GKVK (Gandhi Krishi Vigyan Kendra), Bengaluru-560 065, Karnataka, India, during 2025 to 2026.

Plant Material and Explant Source

Sprouts and nodal segments of the medium-maturing, late blight-resistant potato cultivar 'Kufri Himalini' were used as explants. Sprouts were excised from healthy, disease-free tubers, while single-node segments were collected from pot-grown plants after removing the leaves. The explants were initially washed thoroughly with tap water to remove dirt and debris. A few drops of Tween-20 were added, and the explants were washed under running tap water for 15 to 20 minutes before surface sterilization.

Micropropagation of Plantlets

The explants were surface-sterilized using 2 per cent sodium hypochlorite for 5 minutes and subsequently rinsed 4 to 5 times with sterile distilled water to remove residual sterilant and prevent tissue damage. Shoot regeneration was carried out on Murashige and Skoog (MS) medium supplemented with 30 g sucrose, 6 g agar and different plant-derived extracts like coconut water, potato homogenate, banana homogenate and tomato homogenate at 10, 15 and 20 per cent concentration. The sterilized sprouts and nodal segment explants were inoculated onto the regeneration medium, maintaining the correct polarity of the explants. The cultures were incubated at 25 ± 2°C under a photoperiod of 16 hours of light and 8 hours of darkness, with a light intensity of 3,000 to 3,500 lux. Regenerated shoots measuring 5 to 6 cm in length were transferred to rooting medium containing half-strength MS medium with different plant-derived extracts at 10, 15 and 20 per cent. Before biological hardening, plantlets with well-developed adventitious roots were pre-hardened by removing from the culture vessels and maintaining them in the culture room for one week. This procedure was intended to promote leaf adaptation to lower humidity and water stress through stomatal regulation. The rooted plantlets were subsequently used for the biological hardening experiments (Fig. 1).

Preparation of Bioagents

To enhance the survivability of *in vitro*-raised potato plantlets during the hardening phase, beneficial microbial bioagents, namely *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride*, were used individually and in different combinations. Pure cultures of these bioagents were procured from the Department of Microbiology, University of Agricultural Sciences (UAS), GKVK, Bengaluru. For the preparation of bacterial bioagents, a loopful of inoculum from each pure culture was inoculated separately into 100 mL of nutrient broth in culture flasks. The inoculated flasks were incubated at 28 ± 2°C for 3 to 5 days. Similarly, the fungal bioagent *T. viride* was cultured by inoculating a loopful of fungal inoculum into 100 mL of potato dextrose broth (PDB), followed by incubation at 28 ± 2°C for 3 to 5 days. Before application in the biological hardening treatments, the culture broths of the individual bioagents and their combinations were filtered through filter paper. The resulting filtrates were used for root treatment of *in vitro*-raised potato plantlets during *ex vitro* acclimatization. The different bioagents used in the study and glycerol at a concentration of 0.5 per cent was used as an anti transpirant to reduce transpiration losses and improve the survival of *in vitro*-raised plantlets during the hardening process presented in Table 1 and Fig. 2.

Ex Vitro Acclimatization

Well-rooted *in vitro*-raised potato plantlets were carefully removed from the culture vessels using forceps to avoid mechanical damage. The agar medium adhering to the roots was gently washed off under running tap water to remove residual medium and minimize the risk of contamination. The roots were then dipped in the respective bioagent culture broths for 2 to 3 minutes, according to the treatment. Anti transpirant treatment was provided using 0.5 per cent glycerol, in which the shoot portions, including the stems and leaves, were dipped for five minutes. The treatments were applied according to the experimental treatments, with or without prior bioagent application. Immediately after treatment, the plantlets were transferred to protrays containing sterile cocopeat and maintained in a growth chamber for approximately 15 days for primary hardening. Humidity was maintained by regularly spraying water inside the covering bags to minimize moisture loss and reduce transplanting stress. After primary hardening, the established plantlets were transplanted into pots containing a potting mixture of farmyard manure (FYM), sand and soil in a 1:1:1 ratio for further establishment. Observations on plant survival were recorded at the end of the first, second and third weeks of acclimatization. Plant height, internodal length and root length were measured initially, at the time of bioagent root dipping, and again after three weeks of acclimatization to assess plant growth and establishment under *ex vitro* conditions.

Experimental Design and Statistical Analysis

The *in vitro* microtuberization experiment was conducted in a completely randomized design (CRD) with five replicates and were used to evaluate all of the data. The significance of treatment effects was assessed using the F-test at the 1 per cent level of significance. Statistical analysis was carried out using OPSTAT online software (Sekhar *et al*., 2019).

RESULTS

Effect of different biological hardening treatments on survival of *in vitro* raised plantlets

The effect of different bio-agents, applied individually and in combination with glycerol (0.5 %), on the survival of *in vitro* raised potato plantlets under partially controlled conditions was evaluated after the 1st, 2nd and 3rd weeks of transplanting, as presented in Table 2. The bio-agents were applied as a root dip for 3 minutes, while glycerol (0.5 %) was applied as a shoot and leaf dip for 5 minutes. The observations on plantlet survival were recorded at each interval to assess the effectiveness of the different biological hardening treatments. The results revealed that T_{10} (*Pseudomonas fluorescens* + *Trichoderma viride* + glycerol 0.5 %) recorded the highest plant survival of 93.33, 86.67 and 83.33 per cent after the 1st, 2nd and 3rd week of transplanting, respectively. This was followed by T_5 (*Pseudomonas fluorescens* + *Trichoderma viride*), which recorded 90.00, 83.33 and 80.00 per cent survival, respectively. The third-best treatment was T_6 (*Trichoderma viride* + glycerol 0.5 %), with 86.67, 80.00 and 76.67 per cent survival at the respective intervals. In contrast, the lowest survival was recorded in T_{11} (control), with 63.33, 60.00 and 56.67 per cent, respectively. The superior survival under T_{10} may be attributed to the synergistic effect of *Pseudomonas* and *Trichoderma*, along with glycerol-mediated reduction of transplanting stress and improved acclimatization.

Effect of different biological hardening treatments on plant height of *in vitro* raised plantlets

Significant differences were observed among the treatments with respect to plant height, both initially and after 3 weeks of transplanting (Table 3). Initially, the highest plant height was recorded in T_8 (*Bacillus subtilis* + glycerol 0.5 %) at 10.80 cm, followed by T_5 (*Pseudomonas fluorescens* + *Trichoderma viride*) and T_7 (*Pseudomonas fluorescens* + glycerol 0.5 %), both recording 10.70 cm. After 3 weeks, T_{10} (*Pseudomonas fluorescens* + *Trichoderma viride* + glycerol 0.5 %) recorded the highest plant height (19.03 cm), followed by T_5 (18.17 cm) and T_6 (*Trichoderma viride* + glycerol 0.5 %) (17.13 cm) (Fig. 3). The lowest plant height after 3 weeks was recorded in T_{11} (control) at 14.47 cm. The superior performance of T_{10} may be attributed to the synergistic effects of *Pseudomonas* and *Trichoderma*, along with glycerol, which may enhance nutrient availability, root development and plantlet acclimatization.

Effect of different biological hardening treatments on internodal length of *in vitro* raised plantlets

Significant differences were also observed among the treatments for internodal length, both initially and after 3 weeks of transplanting (Table 4). Initially, the maximum internodal length was recorded in T_1 (*Trichoderma viride*) at 1.67 cm, followed by T_2 (*Pseudomonas fluorescens*) and T_8 (*Bacillus subtilis* + glycerol 0.5 %), both recording 1.63 cm. After 3 weeks, T_{10} (*Pseudomonas fluorescens* + *Trichoderma viride* + glycerol 0.5 %) recorded the highest internodal length (2.05 cm), followed by T_5 (*Pseudomonas fluorescens* + *Trichoderma viride*) (2.02 cm) and T_6 (*Trichoderma viride* + glycerol 0.5%) (2.01 cm). The lowest internodal length was recorded in T_{11} (control) at 1.83 cm. The increased internodal length under T_{10} may be associated with the combined growth-promoting effects of *Pseudomonas*, *Trichoderma* and glycerol, which supported better plantlet growth during acclimatization.

Effect of different biological hardening treatments on root length of *in vitro* raised plantlets

Root length was significantly influenced by the different bio-agents, applied alone or in combination with glycerol (0.5 %) (Table 5). Initially, the highest root length was recorded in T_{10} (*Pseudomonas fluorescens* + *Trichoderma viride* + glycerol 0.5 %) at 7.27 cm, followed by T_5 (*Pseudomonas fluorescens* + *Trichoderma viride*) at 7.17 cm and T_6 (*Trichoderma viride* + glycerol 0.5 %) at 7.10 cm. After 3 weeks of transplanting, T_{10} maintained the highest root length (10.83 cm), followed by T_5 (10.07 cm) and T_6 (9.53 cm). The lowest root length was recorded in T_{11} (control), with 6.27 cm initially and 7.67 cm after 3 weeks. The enhanced root development under T_{10} may be attributed to the synergistic action of *Pseudomonas* and *Trichoderma*, along with glycerol, which may promote root growth, nutrient acquisition and better acclimatization of plantlets.

DISCUSSION

The experiment was conducted to evaluate the effect of different biological hardening treatments on the acclimatization and establishment of micropropagated potato plantlets. Acclimatization is a critical stage in micropropagation because plantlets are sensitive to environmental stresses during transfer from *in vitro* to *ex vitro* conditions (Hazarika *et al*., 2006). In the present study, plant survival gradually decreased from the first to the third week across all treatments. T_{10} (*Pseudomonas fluorescens* + *Trichoderma viride* + 0.5 % glycerol) recorded the highest survival rates of 93.33, 86.67 and 83.33 per cent during the first, second and third weeks, respectively, whereas the control recorded the lowest survival rates of 63.33, 60.00 and 56.67 per cent. The improved survival under T_{10} may be attributed to the complementary effects of the bioagents and the anti transpirant action of glycerol in reducing transplanting stress and excessive water loss (Gawad, 2015). Similar beneficial effects of bioagents and glycerol have been reported by Trivedi and Pandey (2007), Cheng *et al*. (2008), Bhushan and Gupta (2010), Santos *et al*. (2010), Anith *et al*. (2011) and Gupta *et al*. (2017).

Plant height increased in all treatments during the three-week acclimatization period. The maximum plant height was recorded in T_{10} (19.03 cm), followed by T_5 (*P. fluorescens* + *T. viride*) at 18.17 cm and T_6 (*T. viride* + 0.5 % glycerol) at 17.13 cm, while the control recorded the minimum height of 14.47 cm. In T_{10}, plant height increased from 9.90 to 19.03 cm. The superior growth may be associated with improved nutrient availability, root development and plant-growth-promoting activities of the bioagents. The growth-promoting effects of *Trichoderma* species have been associated with

the production or modulation of auxins, gibberellins and other physiological compounds (Montealegre *et al*., 2010; Druzhinina *et al*., 2011; Brotman *et al*., 2013). Similar beneficial effects of microbial treatments on micropropagated potato and banana have been reported by Lavanya *et al*. (2009), Lincy and Sasikumar (2010), Sparta and Emilda (2020) and Naik *et al*. (2025).

Internodal length also increased during acclimatization, with the maximum value recorded in T_{10} (2.05 cm), followed by T_5 (2.02 cm) and T_6 (2.01 cm). The control recorded the minimum internodal length of 1.83 cm. The increase in internodal length in T_{10} from 1.64 to 2.05 cm suggests improved shoot elongation under the combined treatment. This response may be related to the plant-growth-promoting effects of *P. fluorescens* and *T. viride*, including improved nutrient uptake and the possible modulation of growth-regulating compounds such as auxins and gibberellins (Harman *et al*., 2004; Druzhinina *et al*., 2011). These findings indicate that the combined application of bioagents and glycerol may support vegetative growth during the hardening process.

Root development was also enhanced by the biological hardening treatments. T_{10} recorded the maximum root length of 10.83 cm, followed by T_5 (10.07 cm) and T_6 (9.53 cm), whereas the control recorded the minimum root length of 7.67 cm. In T_{10}, root length increased from 7.27 to 10.83 cm. The improved root growth may be attributed to the complementary effects of *P. fluorescens* and *T. viride* in promoting root development and nutrient uptake, while glycerol may help reduce moisture stress during acclimatization. Similar beneficial effects of microbial inoculation on plant growth and root development have been reported in *Azadirachta indica* (Lavanya *et al*., 2009), ginger (Lincy and Sasikumar, 2010), banana (Sparta and Emilda, 2020) and potato (Naik *et al*., 2025). Overall, T_{10} was the most effective treatment, recording the highest survival, plant height, internodal length and root length after three weeks, demonstrating its potential for improving the acclimatization and establishment of micropropagated potato plantlets.

CONCLUSION

Overall, T_{10} (*P. fluorescens* + *T. viride* + 0.5 % glycerol) was the best-performing treatment across all four parameters after three weeks of acclimatization, recording the highest plant survival (83.33 %), plant height (19.03 cm), internodal length (2.05 cm) and root length (10.83 cm). It was followed by T_5 (*P. fluorescens* + *T. viride*), while T_6 (*T. viride* + 0.5 % glycerol) also showed comparatively favourable results. The control consistently recorded lower values for survival and growth parameters. The superior performance of T_{10} suggests that the combined application of *P. fluorescens*, *T. viride* and glycerol may have contributed to improved acclimatization, root establishment and vegetative growth through complementary microbial and anti transpirant effects. Thus, the integration of beneficial bioagents with glycerol appears to be a promising approach for improving the survival and establishment of micropropagated potato plantlets during *ex vitro* hardening.

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Fig. 1. The rooted plantlets were subsequently used for the biological hardening experiments



Fig. 2. Biological hardening of *in vitro* raised potato plantlets using bio-agents: (a) *Pseudomonas fluorescens*, (b) *Bacillus subtilis*, (c) *Trichoderma viride*, (d) *Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5 %), (e) Glycerol (0.5 %) - root dip, and (f) Glycerol (0.5 %) - shoot dip

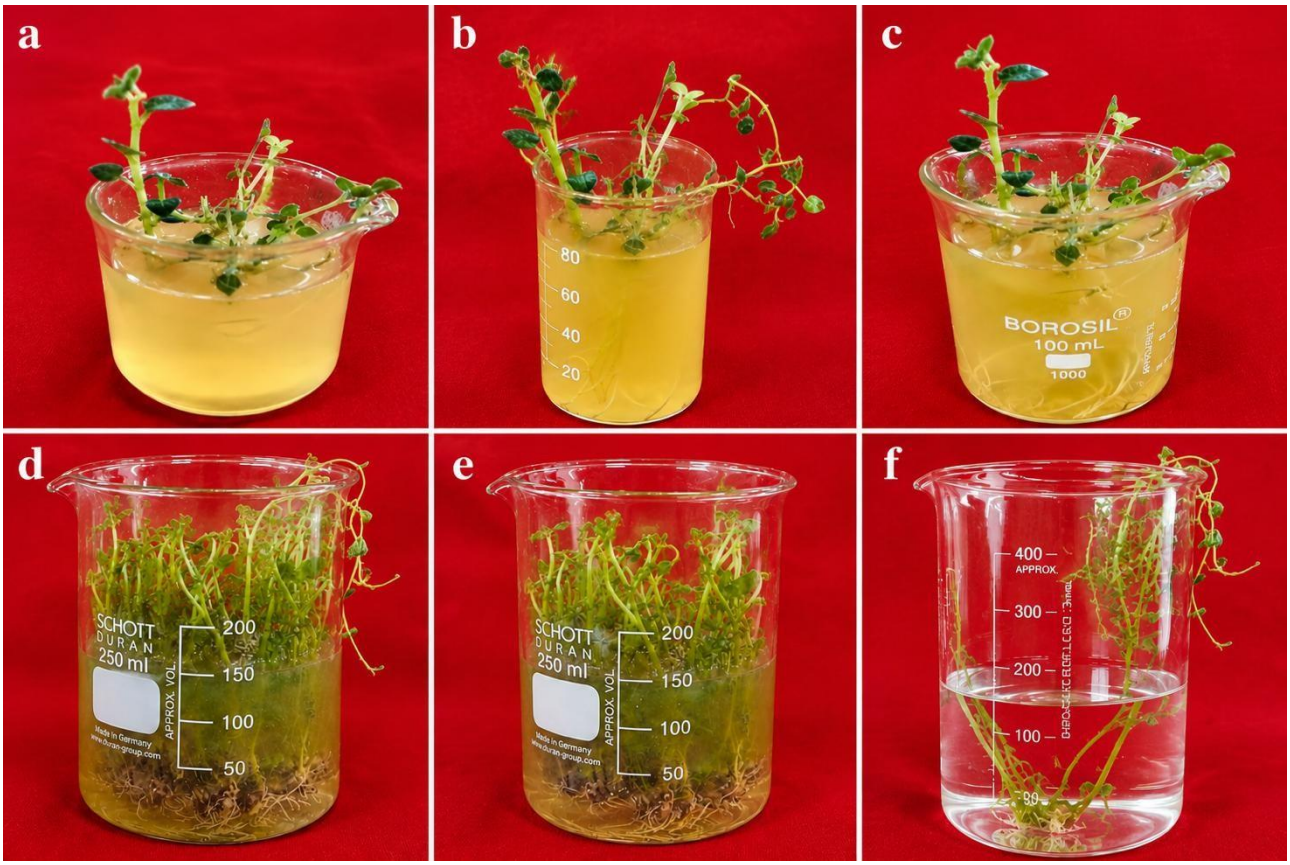

Fig. 3. Effect of bio-agents on plant height after 3 weeks of hardening: (a) Control, (b) *Trichoderma viride* + Glycerol (0.5 %), (c) *Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5 %), and (d) *Pseudomonas fluorescens* + *Trichoderma viride*



Table 1. Effect of biological hardening treatments on *in vitro* raised potato plantlets

Treatments	**Combinations**
T_1	*Trichoderma viride*
T_2	*Pseudomonas fluorescens*
T_3	*Bacillus subtilis*
T_4	*Pseudomonas fluorescens* + *Bacillus subtilis*
T_5	*Pseudomonas fluorescens* + *Trichoderma viride*
T_6	*Trichoderma viride* + Glycerol (0.5 %)
T_7	*Pseudomonas fluorescens* + Glycerol (0.5 %)
T_8	*Bacillus subtilis* + Glycerol (0.5 %)
T_9	*Pseudomonas fluorescens* + *Bacillus subtilis* + Glycerol (0.5 %)
T_{10}	*Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5 %)
T_{11}	Control (seedling dip in distilled water)

Table 2. Effect of bio-agents on the survival percentage of *in vitro* raised potato plantlets

Treatments		**Plant survival rate (%)**		
		After 1st week	**After 2nd week**	**After 3rd week**
T_1	*Trichoderma viride*	83.33	76.67	70.00
T_2	*Pseudomonas fluorescens*	73.33	70.00	66.67
T_3	*Bacillus subtilis*	66.67	63.33	60.00
T_4	*Pseudomonas fluorescens* + *Bacillus subtilis*	80.00	73.33	70.00
T_5	*Pseudomonas fluorescens* + *Trichoderma viride*	90.00	83.33	80.00
T_6	*Trichoderma viride* + Glycerol (0.5 %)	86.67	80.00	76.67
T_7	*Pseudomonas fluorescens* + Glycerol (0.5 %)	76.67	73.33	70.00
T_8	*Bacillus subtilis* + Glycerol (0.5 %)	73.33	70.00	63.33
T_9	*Pseudomonas fluorescens* + *Bacillus subtilis* + Glycerol (0.5 %)	80.00	73.33	66.67
T_{10}	*Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5 %)	93.33	86.67	83.33
T_{11}	Control (seedling dip in distilled water)	63.33	60.00	56.67
***F*-Test**		**	**	**
SEm ±		**0.28**	**0.27**	**0.25**
CD at 1 %		**1.13**	**1.06**	**0.98**

Table 3. Effect of bio-agents on the plant height of *in vitro* raised potato plantlets

Treatments		**Plant height (cm)**	
		Initial length	**After 3 weeks**
T_1	*Trichoderma viride*	9.70	16.97
T_2	*Pseudomonas fluorescens*	10.40	15.67
T_3	*Bacillus subtilis*	10.30	15.07
T_4	*Pseudomonas fluorescens* + *Bacillus subtilis*	10.50	16.17
T_5	*Pseudomonas fluorescens* + *Trichoderma viride*	10.70	18.17
T_6	*Trichoderma viride* + Glycerol (0.5%)	10.00	17.13
T_7	*Pseudomonas fluorescens* + Glycerol (0.5%)	10.70	16.00
T_8	*Bacillus subtilis* + Glycerol (0.5%)	10.80	15.17
T_9	*Pseudomonas fluorescens* + *Bacillus subtilis* + Glycerol (0.5%)	10.30	16.80
T_{10}	*Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5%)	9.90	19.03
T_{11}	Control (seedling dip in distilled water)	10.10	14.47
***F*-Test**		**	**
SEm ±		**0.13**	**0.74**
CD at 1 %		**0.53**	**2.96**

Table 4. Effect of bio-gents on the internodal length of *in vitro* raised potato plantlets

Treatments		**Internodal length (cm)**	
		Initial length	**After 3 weeks**
T_1	*Trichoderma viride*	1.67	1.96
T_2	*Pseudomonas fluorescens*	1.63	1.90
T_3	*Bacillus subtilis*	1.60	1.85
T_4	*Pseudomonas fluorescens* + *Bacillus subtilis*	1.47	1.93
T_5	*Pseudomonas fluorescens* + *Trichoderma viride*	1.60	2.02
T_6	*Trichoderma viride* + Glycerol (0.5%)	1.57	2.01
T_7	*Pseudomonas fluorescens* + Glycerol (0.5%)	1.57	1.91
T_8	*Bacillus subtilis* + Glycerol (0.5%)	1.63	1.87
T_9	*Pseudomonas fluorescens* + *Bacillus subtilis* + Glycerol (0.5%)	1.57	1.94
T_{10}	*Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5%)	1.64	2.05
T_{11}	Control (seedling dip in distilled water)	1.53	1.83
***F*-Test**		**NS**	**
SEm ±		**0.08**	**0.03**
CD at 1 %		**0.34**	**0.11**

Table 5. Effect of bio-gents on the root length of *in vitro* raised potato plantlets

Treatments		**Root length (cm)**	
		Initial length	**After 3 weeks**
T_1	*Trichoderma viride*	6.83	9.33
T_2	*Pseudomonas fluorescens*	6.53	8.53
T_3	*Bacillus subtilis*	6.33	8.03
T_4	*Pseudomonas fluorescens* + *Bacillus subtilis*	6.83	9.07
T_5	*Pseudomonas fluorescens* + *Trichoderma viride*	7.17	10.07
T_6	*Trichoderma viride* + Glycerol (0.5%)	7.10	9.53
T_7	*Pseudomonas fluorescens* + Glycerol (0.5%)	6.80	8.67
T_8	*Bacillus subtilis* + Glycerol (0.5%)	6.40	8.50
T_9	*Pseudomonas fluorescens* + *Bacillus subtilis* + Glycerol (0.5%)	6.80	9.23
T_{10}	*Pseudomonas fluorescens* + *Trichoderma viride* + Glycerol (0.5%)	7.27	10.83
T_{11}	Control (seedling dip in distilled water)	6.27	7.67
***F*-Test**		**	**
SEm ±		**0.11**	**0.43**
CD at 1 %		**0.43**	**1.71**