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Effect of microbial-inoculants on growth and biochemical parameters of mango plantlets during bio-hardening

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ABSTRACT

Present investigation was carried out to find out the effects of bio-agents on growth and biochemical changes during bio-hardening of *in vitro* embryo cultured plantlets of mango genotypes Amrapali, Pusa Arunima and hybrid 8-11. *In vitro* raised plantlets were subjected to bio-hardening using three microbial, *Aspergillus niger* and microbial consortium (mixed AMF strains, Nutrilink®). Plants treated with bio-inculants had 4.8 to 19.98 per cent more plant height compared to control. Similarly, root length, fresh weight, dry weight, leaf number and secondary roots were significantly increased by mycorrhizal inoculation compared to control plants. The histo-chemical studies revealed that the mycorrhizal inoculation resulted in enhanced accumulation of different metabolites such as chlorophyll, total phenols, total sugars, while the proline accumulation was reduced during bio-hardening. The poly-phenol oxidase enzymes like catacholase and cresolase activities were enhanced by microbe inoculation. The mycorrhizal plantlets showed enhanced survival and improved tolerance against stresses experienced during acclimatization phase.

Key words: Bio-hardening, *in vitro in-ovulo* embryo culture, mango, mycorrhizal inoculation.

INTRODUCTION

Mycorrhizal associations vary widely in structure and function, but the most common interaction is the arbuscular mycorrhizal (AM) symbiosis. This interaction is formed between the roots of over 80% of all terrestrial plant species. These fungi are termed AM fungi and are obligate symbionts which form endomycorrhizal symbioses. Filamentous fungi are widely used as producers of organic acids, particularly black *Aspergillus* and some species of *Penicillium*, these species have been tested for solubilization (Yadav *et al.*, 15) and have been reported for various properties of biotechnological importance, such as, biocontrol, biodegradation, phosphate solubilization (Pandey *et al*., 8). Survival of *in vitro* raised mango plantlets can be effectively increased by using bio-agents known to have symbiotic association with roots and found to be beneficial. This symbiotic relationship stimulates growth and development of plantlets by increasing the absorption of water and immobile nutrients and also increases drought tolerance and minimise disease incidence. There are a few reports of the response of mango crop to AM fungal inoculation. Recent studies have also shown that root longevity, morphology and structure of host plants are affected by mycorrhization (Hodge *et al*., 4). The present investigation was conducted to find the biochemical changes brought about by bio-agents inoculation in order to understand the possibility of existence of other mechanism for enhanced plant survival during bio-hardening.

MATERIALS AND METHODS

The present study was carried out on *in vitro in-ovulo* embryo cultured plantlets of mango genotypes namely, Amrapali, Hybrid 8-11 and Pusa Arunima at the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi. The *in vitro* raised embryo cultured plantlets were subjected to bio-agent treatments during hardening. Cultures of arbuscular mycorrhizal fungi (AMF), namely, *Glomus intraradices* (T1), *Gigaspora margarita* (T2), *Glomus fasciculatum* (T3), Nutrilink® (T4, obtained from Division of Microbiology, ICAR-IARI, consortium of *Glomus mosseae*, *G. fasciculatum* and *Gigaspora margarita*) and *Aspergillus niger* (AN-27) (T5) were used in cocopeat + perlite medium for bio-hardening of embryo cultured mango plants. Pure cultures of arbuscular mycorrhizal fungi (AMF) and *Aspergilus niger* were procured from Division of Microbiology and Division of Pathology ICAR-IARI, New Delhi. Bio-agent treatments consisted of approximately 20 g inoculum (containing rhizosphere soil, spores besides hyphae, arbuscules and vesicles (for AMF) and root segments of *Bahia* grass). The soil based AMF cultures were maintained under glasshouse conditions in plastic pots containing *Bahia* grass (*Paspalum notatum*) grown on sterile potting mixture (Soil: sand: FYM, 2:2:1). Potting mixture was sterilized

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in autoclave (Sanco Pvt. Ltd.) at 121°C and 151 lb/in^2. Microbial treatments consisted spores besides hyphae, arbuscules and vesicules (of AMF) and root segments of host plant, *i.e*. Bahia grass.

Acclimatization of *in vitro* raised mango plantlets was done by washing the roots with sterile deionized water to dislodge the adhering agar-agar and then potting into mixture consisting of sterile soil, sand and FYM (2:2:1) in jam bottle and further in plastic pots (5 inch dia.). These pots were further transferred in glass house and plantlets were maintained in glasshouse with day-night temperatures ranging from 27 ± 2°C. Day length was extended to 16 h with cool white fluorescent lights at 630 µmol sec^{-1}m. Humidity was maintained to the level of 85 per cent using humidifier. Plantlets were watered on alternate days with sterile tap water.

Plant height (cm), fresh weight (g), dry weight (g), number of secondary roots, leaf number and root length were recorded 45 days after inoculation. Biochemical parameters, *viz.,* total sugars, polyphenol oxidase activity, proline content, total phenols and total chlorophyll were observed after 30 and 60 days of inoculation. The total soluble sugars was estimated according to the anthrone reagent method (Hedge and Hofreiter, 3). Both catecholase and cresolase activities were measured employing the method suggested by Sanchenz-Ferrer *et al*. (9) and Valero *et al*. (13) with some modifications. Leaf chlorophyll content (chlorophyll a, b and total chlorophyll) was determined from fully matured leaves according to Barnes *et al*. (1).

The experiments were laid out in completely randomized design. ANOVA was calculated to partition the variance as reported by Gomez and Gomez (2). Valid conclusions were drawn only on significant differences between the treatment mean at 0.05 level of probability.

RESULTS AND DISCUSSION

AMF inoculation at the acclimatization stage increased the growth of morphological parameters *viz.,* plant height, fresh weight, dry weight, number of secondary roots, leaf number and root length. It might be due to beneficial effect of mycorrhization in providing more water and nutrient absorption helping the plantlets in better adapting to transplanting shock and root diseases. After 45 days after inoculation, plantlets inoculated with Nutrilink® showed maximum plant growth, while among genotypes maximum growth was observed in Pusa Arunima (Tables 1 & 2). The results of the present investigation are in corroboration with the findings of Sharma *et al*. (10) and Singh *et al*. (11). The increased growth might be due to better mobilization of various essential nutrient and water. Yadav *et al*. (16) also found that combination of mycorrhizal species had better effect on survival and growth of *Gloriosa superba*.

Inoculation of AMF induced significant impacts on biochemical attributes of *in vitro* raised plantlets. Consortium of *Glomus mosseae, Glomus fasciculatum* and *Gigaspora margarita* (AMF) inoculated plantlets showed maximum total sugars (4.56 mg g^{-1} FW), which was significantly higher over the control. In all three mango genotypes, the level of total sugars gradually increased during bio-hardening. The maximum total sugars was noted in Pusa Arunima plants grown on medium having consortium of AMF. Whereas, the minimum total sugars was observed in Amrapali plants without bio-agents treatments (control) (Fig. 1A). The result of present investigation is in corroboration with the findings of Wu and Xia (14) who found that soluble sugars were increased by AM inoculation.

Total phenols content was maximum in *Glomus intraradices* treated plants, which was significantly higher than rest of the bio-agent treatments and control. Whereas, minimum phenol content was observed in control plants, which was statistically at par with the *Gigaspora margarita*, microbial consortium and *Aspergillus niger* treatments (Fig. 1B). In the present investigation, all the microbial treatments were found to enhance the level of phenols in all mango genotypes during all stages of sampling. This result is in conformity with the findings of Krishna *et al*. (5) on grape. It was also observed in the present study that PPO activity was enhanced by microbial inoculation, which might be responsible for enhanced phenolic contents in the treated plants.

The proline content was recorded to be maximum in Pusa Arunima plants (23.35 µg/g) without bio-agent treatment, however minimum was in *Glomus intraradices* treated hybrid H-8-11(16.27 µg/g) plants. In the present investigation, the mycorrhizal plants showed lowered proline content in all mango genotypes (Fig. 1C). These results are in accordance with the findings of Tholkappian *et al*. (12) who reported lower level of proline in mycorrhizal lettuce and soybean plants. On the contrary, Krishna *et al*. (5) reported higher proline accumulation as a result of VAM treatments.

The catacholase activity significantly differed and found to be the maximum in plants treated with microbial consortium, regardless of mango genotypes. The maximum catacholase activity was observed in Pusa Arunima plants with consortium of *Glomus mosseae, Glomus fasciculatum and Gigaspora margarita* (AMF) bio-agents during bio-hardening. Whereas, the minimum catecholase activity was recorded in hybrid H-8-11 without bio-

Table 1. Effect of bio-agents on plant height, root length and fresh weight of *in vitro* raised mango plantlets during bio-hardening (45 DAI).

Treatment	Plant height (cm)				Root length (cm)				Fresh wt. (g)			
	Amrapali	Pusa Arunima	H-8-11	Mean	Amrapali	Pusa Arunima	H-8-11	Mean	Amrapali	Pusa Arunima	H-8-11	Mean
Control	10.39	10.48	10.37	10.41	6.36	6.52	6.24	6.37	6.26	6.36	6.14	6.25
Glomus intraradices	12.05	12.10	11.77	11.97	7.67	7.78	7.57	7.67	6.96	7.02	6.90	6.96
Gigaspora margarita	11.02	11.37	10.33	10.91	6.50	6.81	6.47	6.59	6.29	6.31	6.25	6.28
Glomus fasiculatum	11.34	11.53	11.23	11.37	7.00	7.08	6.80	6.96	6.33	6.38	6.28	6.33
Nutrilink® (microbial consortium)	12.28	13.00	12.20	12.49	8.47	8.57	8.40	8.48	7.09	7.13	7.07	7.10
Aspergillus niger	10.72	11.40	10.87	11.00	6.88	6.90	6.77	6.85	6.38	6.43	6.33	6.38
Mean	11.30	11.65	11.13	-	7.15	7.27	7.04	-	6.55	6.60	6.49	-
CD at 5%												
Treatment (T)		1.04				1.05				0.49		
Genotype (G)		0.73				0.74				0.35		
T × G		1.80				1.81				0.85		

Table 2. Effect of bio-agents on dry weight, number of secondary roots and leaves of *in vitro* raised mango plantlets during bio-hardening (45 DAI).

Treatment	Dry wt. (g)				No. of secondary roots				No. of leaves			
	Amrapali	Pusa Arunima	H-8-11	Mean	Amrapali	Pusa Arunima	H-8-11	Mean	Amrapali	Pusa Arunima	H-8-11	Mean
Control	1.55	1.58	1.54	1.56	6.57	6.80	6.50	6.62	5.20	5.33	5.31	5.28
Glomus intraradices	1.68	1.74	1.60	1.67	9.00	9.47	8.99	9.15	5.42	5.56	5.46	5.48
Gigaspora margarita	1.61	1.62	1.59	1.60	7.75	7.78	7.67	7.73	4.88	4.94	4.83	4.88
Glomus fasiculatum	1.68	1.70	1.65	1.68	7.00	7.37	6.97	7.11	5.00	5.08	5.08	5.06
Nutrilink® (microbial consortium)	1.88	1.90	1.85	1.88	9.50	9.70	9.47	9.56	5.34	5.17	5.20	5.59
Aspergillus niger	1.53	1.56	1.52	1.54	7.33	7.45	7.29	7.36	5.24	5.16	5.09	5.16
Mean	1.66	1.68	1.63	-	7.86	8.10	7.81	-	5.18	5.20	5.24	-
CD at 5%												
Treatment (T)		0.16				0.60				0.24		
Genotype (G)		0.11				0.42				0.17		
T × G		0.27				1.03				0.42		

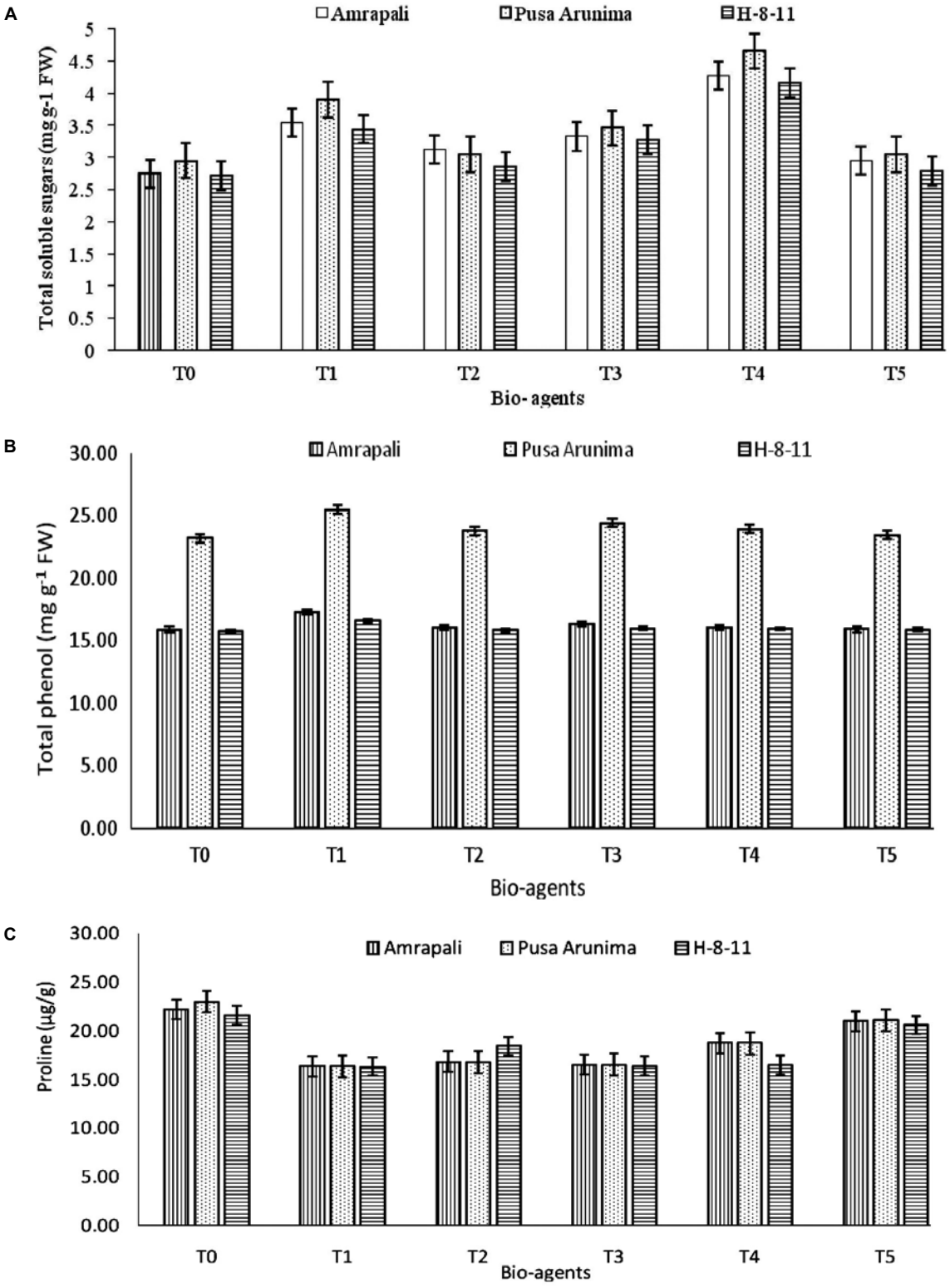

Fig. 1. Effect of bio-agent treatments on A) total sugars, B) total phenols and C) proline contents of *in vitro in ovulo* embryo cultured mango plantlets during bio-hardening. T0, Control; T1, *Glomus intraradices*; T2, *Gigaspore margarita*; T3, *Glomus fasiculatum*; T4, Nutrilink (microbial consortium); T5, *Aspergillus niger*. Vertical bars indicate ± SE of means.

agents treatment. Whereas, cresolase activity was found to be the maximum in *Gigaspora margarita* treatment, regardless of mango genotypes and duration of bio-hardening. The maximum cresolase activity was observed in Pusa Arunima plants grown on *Gigaspora margarita* during bio-hardening. Whereas, the minimum cresolase activity was recorded in hybrid H-8-11 during without bio-agents treatment (Fig. 2A & B). The increased PPO activity might be responsible for increased phenolic contents in the plants. Present results are also supported by the findings of Yadav *et al.* (16).

All AMF treated plantlets showed higher chlorophyll content over control. The maximum chlorophyll a, b and total chlorophyll were noted in *Glomus intraradices* treated plants and minimum was observed in control plants. In general, the mycorrhizal inoculated plants had more photosynthetic pigments as compared to control. The *Aspergilus niger* treatment plants showed non-significant differences for photosynthetic pigments compared to control (Fig. 3A, B & C). This result is in conformity with the findings of Kumar *et al.* (6), Yadav *et al.* (16) and Neelkandan and Mahesh (7), who reported increased chlorophyll contents in leaves as a result of AMF inoculation. The increased chlorophyll content in leaves could be attributed to enhanced uptake of nutrients which are essential for chlorophyll synthesis (Sharma *et al*., 10).

This study suggests that AMF strains improved the morphological and biochemical attributes which are necessary to mitigate adverse effects of transplanting shock and enhancing survival. Among bio-agents, consortium of AMF followed by *Glomous intraradices* were found superior suggesting their high suitability as bio-hardening agents for *in-vitro* embryo raised mango plantlets.

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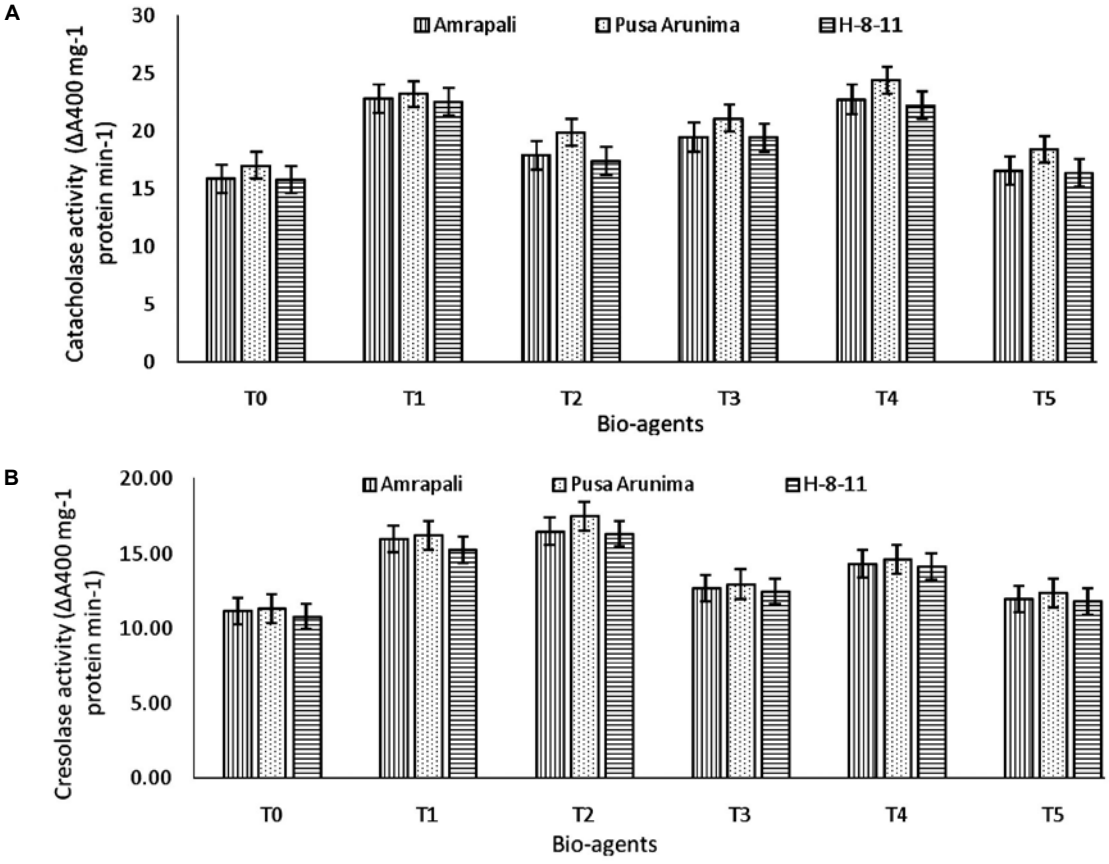

Fig. 2. Effect of bio-agents treatment on A) catacholase and B) cresolase activities of *in vitro in ovulo* embryo cultured mango plantlets during bio-hardening. T0, Control; T1, *Glomus intraradices*; T2, *Gigaspore margarita*; T3, *Glomus fasiculatum*; T4, Nutrilink (microbial consortium); T5, *Aspergillus niger*. Vertical bars indicate ± SE of means.

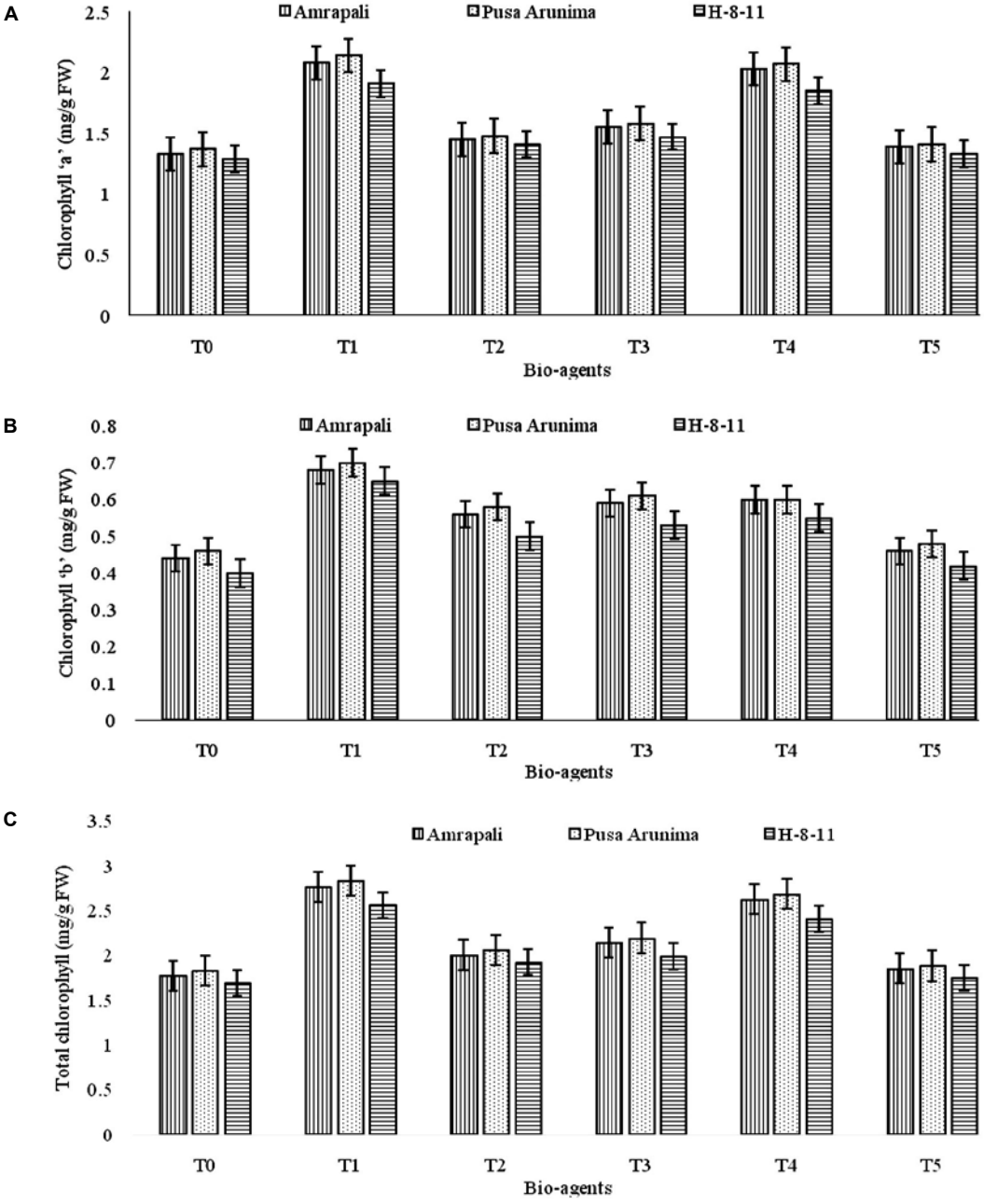

Fig. 3. Effect of bio-agents on A) chlorophyll a, B) cholorophyll b and C) total cholophyll contents of *in vitro in ovulo* embryo cultured mango plantlets during bio-hardening. T0, Control; T1, *Glomus intraradices*; T2, *Gigaspore margarita*; T3, *Glomus fasiculatum*; T4, Nutrilink (microbial consortium); T5, *Aspergillus niger*. Vertical bars indicate ± SE of means.

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