The Effects of Bioinoculants on Enhancement Growth in NanjanagudRasabale Tissue Culture Banana

Abstract

Bio-hardening is a process where micro propagated plantlets are inoculated with suitable strains of microorganisms in order to enhance their acclimatization. Trials were taken to expose banana plantlets to useful microbes that encourage growth and mutual association during2022 at UHS,Bagalkot.It promotes host plant growth, plant nutrient uptake, inhibits pathogen growth, reduces disease severity, enhances tolerance to environmental stress *etc.* In the present study, twenty treatments were imposed with different bioinoculants by two methods of application (1) powder formulation and (2) liquid formulation. Among these treatments, M_1T_{13}- VAM and fusicont significantly increased the survivabilityper cent (58.80%), plant height(6.46 cm), pseudo stem girth (5.42 cm), number of leaves (4.07) and leaf area (33.15 cm^2). Thus, the artificial inoculation of bioinoculants through the powder formulation enhances the growth during primary hardening.

Keywords: Artificial inoculation, methods of application, tissue culture banana.

1. INTRODUCTION

The banana is one of the oldest fruits known to humankind and the second most important fruit crop in India after the mango. Millions of people in the tropics eat bananas for food (Bakry *et al.*, 2009). India alone, among the largest producers, occupies an area of 8,83,000 ha, produces 308,000 MT, and has a productivity of 34.9 MT/ha (Anonymous, 2018). Due to their parthenocarpy nature and sterile seed, sucker propagation is the only natural means for their perpetuation (Singh *et al.*, 2011). Bananas are also facing many problems by biotic and abiotic stresses. So,bio hardening of plantlets before planting in the main field increases its acclimatization in field condition.

Using bio-hardening during the acclimatization phase can increase growth and reduce mortality. Trials to expose plantlets to beneficial microbes that encourage growth and interplant communication have been done. It increases resistance to environmental stress, promotes the growth and uptake of nutrients by the host plant, inhibits the growth of pathogens, and lessens the severity of disease. Bioagents produce siderophores, phytohormones like auxin (IAA), produce hydrolytic enzymes, exo-polysaccharides, solubilize phosphorus and potassium, fix nitrogen, release auxin (IAA), and induce systemic resistance. The relationship between the bioagent and the host has frequently been seen as a mutualistic relationship (Kloepper *et al.*, 1989, Jefwa *et al.*, 2010). With this background, the

investigation entitled "Effect of bioinoculants on enhancement of growth in tissue culture banana *cv*.NanjanagudRasabale during primary hardening"was carried out using two methods of application cocopeat mixing of powder formulation and root dip in liquid formulation of bioinoculants.

2. MATERIAL AND METHODS

The current research was conducted in 2021-2022, at the Centre for Horticulture Biotechnology, Directorate of Research, University of Horticultural Sciences, Bagalkot, to determine the impact of bio-agent inoculation on enhancing shoot and root growth of tissue culture propagated banana plantlets during primary hardening.

NanjangudRasabaletissue cultured plantlets where imposed with Bioagents like *Trichoderma harzianum* (COHF1), *Trichoderma asperulum* (COHF2 & COHF3) which was procured from COH, Bengaluru. *Pseudomonas fluorescens, Bacillus* are directly involved in the reduction of plant pathogens and induce plant resistance (Gray and Gerdemann, 1969). This bioagent was collected from COH, Bagalkot. Based on the effectiveness in the control of the disease under pot and sick field experiments a bio-preparation ICAR-FUSICONT was developed using growth promoting antagonistic agents *Trichoderma reesei* CSR-T-3 (MH997668.1) (Damodaran *et al.,* 2019) and *Lysnibacillus fusiformis* CSR-A-11 (KU745624.1). This fusicont is procured from the central soil salinity research institute (CSSRI), RRS, Lucknow.

The experiment was conducted in polytunnel were possible to manually open and close vents or use equipment mounted inside the polytunnel to regulate the temperature (25°C), humidity (90%) and ventilation. There were 20 treatments made up of various levels in the experiment, which was set up in a Factorial Completely Randomized Design with five replications. Two level M_1cocopeat mixing with powder formulation of bioinoculants and M_2 root dip liquid formulation of bioinoculants methods. There were forty treatment combinations in this experiment of which the first twenty treatments were imposed to powder formulation and the remaining to liquid formulation. The treatment included different combinations of *Trichoderma harzianum* (COHF1),*Trichoderma asperulum*(COHF2 & COHF3), *Pseudomonas fluorescens, Bacillus subtilis,* VAM and Fusicont. B_1: *Trichoderamaharzianum*(COHF1), B_2: *Trichoderamaasperulum*(COHF2), B_3: *Trichoderamaasperulum*(COHF3), B_4: *Pseudomonas fluorescens,* B_5: *Bacillus subtilis,* B_6: VAM, B_7: Fusicont, B_8: VAM+ *Trichoderama harzianum* (COHF1), B_9: VAM+ *Trichoderama asperulum* (COHF2), B_{10}: VAM+ *Trichoderama asperulum* (COHF3), B_{11}: VAM+ *Pseudomonas fluorescens,* B_{12}: VAM+ *Bacillus subtilis,* B_{13}: VAM+Fusicont,B_{14}:

VAM+ *Trichoderama harzianum* (COHF1) + *Pseudomonas fluorescens,*B_{15}: VAM+ *Trichoderama harzianum* (COHF1) + *Bacillus subtilis,* B_{16}: VAM+ *Trichoderama asperulum* (COHF2) + *Pseudomonas fluorescens,* B_{17}: VAM+ *Trichoderama asperulum* (COHF2) + *Bacillus subtilis,* B_{18}: VAM+ *Trichoderama asperulum* (COHF3) + *Pseudomonas fluorescens,* B_{19}: VAM+ *Trichoderama asperulum* (COHF3) + *Bacillus subtilis,* B_{20}: Untreated control (Fig .1)

Before moving the plantlets, formaldehyde was used to fumigate the greenhouse. The media for primary hardening was a blend of cocopeat and perlite. It was wetted, squeezed to remove extra water, and then filled into protrays. During initial hardening, the bio-agents needed for each treatment were weighed and added to the media, mixed well and filled in portrays for powder formulation. The plantlets where dipped in liquid bioinoculants for 1 hour before planting in the portrays (Fig. 2).

They were immediately placed into protrays and given the treatment described above. To all plantlets, a 0.5 g/l foliar treatment of 19:19:19 - N:P:K was applied at intervals of 15 days. Plantlets were kept in a polytunnel at a temperature of 30°C, 90 per cent relative humidity and diffused sunshine.

3. RESULTS AND DISCUSSION

The study demonstrates notable variations among different bioinoculants treatment andbetween two methods of application. Significantly the highest survival rate (50.00%) was recordedwhen the plantlets were treated with the treatment combination VAM+Fusicont (Fig .3). The maximum survival rate and was reported in cocopeat mixing (23.22%) as opposed to root dip (12.24%), The combination of VAM+Fusicont in a cocopeat mixing powder formulation had the highest survival rate of 58.80%. here the cocopeat mixing powder formulation performed well when compared to root dip of liquid formulation as it is due to the factthat the bioagentswhen incorporated in rooting medium was more effective as its multiplication and colonization in rhizosphere will be higher as they are soil inhabitant bioagents (Ramanujam *et al*., 2010). The highest survival rate recorded 58.80% as the polyhousetemperature exceeded up to 47º c during April and May so the sporulation and multiplication decreased in medium which directly affect the survival and growth of plantlets as explained by (Singh *et al*., 2012; Aulakh *et al*., 2017) (Table 1.)

Significantly the maximum pseudostem height, girth, number of leaves, leaf area, chlorophyll was recorded in treatment VAM+Fusicont(5.05 cm,4.46 cm, 3.70, 3.70 cm^2, 37.52 SPAD units respectively) and in method of inoculation highest was recorded with

cocopeat mixing (3.10 cm, 1.19 cm, 2.73, 14.11 cm^2, 34.43 SPAD unitsrespectively) compared with the root dip (1.81 cm, 0.05 cm, 1.75, 7.18 cm^2, 24.84 SPAD unitsrespectively) (Table 2.)

Among the various interaction effect cocopeat mixing with the treatment combination VAM+Fusicont (6.46 cm, 5.42 cm, 4.07, 33.15 cm^2, 40.07 SPAD unitsrespectively)exhibited highest pseudo stem height, girth, number of leaves, leaf area (cm^2) and chlorophyll content (Ikram *et al*., 2019). The VAM fungal hyphae transport phosphate over large distance into the root cortical cells (Beers *et al*., 1952; Singh *et al*., 2010). where, it helps for higher nutrient uptake from the soil to the plant. It has been reported that the effect of VAM fungi is increased when they were inoculated with other micro flora of rhizosphere of plants (Prakash*et al*., 2011). The treatment Fusicont significantly increased the plant height where they are made of *Trichoderma reesei*(soil-borne filamentous fungi) has been known for its potential to help host plant species against biotic and abiotic stresses and they can secrete indole-3-acetic acid (IAA) to control various aspects of plant growth.

According to the data in Table 3, the combination of bioinoculants significantly increased the number of primary roots per plantlet with the treatment combination VAM+ Fusicont (5.57)and Fusicont in secondary roots, length of longest primary roots (16.50), (19.52 cm). And in method of bioinoculants the maximum root parameters was recorded in cocopeat mixing powder formulation (3.98, 14.19, 15.84 cm respectively) compared with root dip liquid formulation (2.41, 6.25, 5.87 cm respectively). Among the various interaction effect cocopeat mixing with the treatment combination VAM+Fusicont in number of primary roots and Fusicont in secondary and length of longest primary root were recorded maximum (6.40, 21.93, 27.87 cm respectively). It might be due to bioagents when applied in media inoculum is able to colonize, establish, compete and survive in the complex soil environment (Mougy*et al*., 2012). The root dip methods showed significantly minimum root growth when compared with the cocopeat mixing it might be due to less colonization and spore formation of bioinoculants and less association in the root zone were the development of primary, secondary and growth of the roots are affect which directly reduces the nutrient uptake (Ramanujam *et al*., 2010).

The treatment combination of VAM+Fusicont was recorded maximum shoot to root ratio of fresh weight and dry weight (14.80 and 10.50 respectively). The maximum shoot to root ratio of fresh weight and dry weight the cocopeat mixing powder formulation method of bioinoculants recorded with (7.21 and 4.87 respectively) compared to root dip (3.6 and 3.50 respectively). Among the various interaction effect cocopeat mixing with the treatment

combination VAM+Fusicont (Fig 3.) noted highest in shoot to root ratio of fresh weight and dry weight (18.20 and 12.77 respectively).

When VAM treated with other bioagents (*Bacillus*, *Trichoderma*spp), increases the root colonization and nutritient uptake as it increases the yield of the plant where it contribute for the dry matter content of the inoculated plants (Singh *et al.*, 2010, Harman *et al.*, 2004).

4. CONCLUSION

Result of the "Effect of bioinoculants on enhancement of growth in tissue culture banana *cv.* NanjanagudRasabale during primary hardening"by applying two methods of application in which the cocopeat mixing powder formulation can be judiciously used for biohardening. Among various bioagents used, the treatment combination of VAM+ Fusicont (T_{13}), Fusicont (T_7) induced the earliest growth and development of the plantlets. It can be concluded that these results would significantly enhance the acclimatization, growth, and nutrient uptake of *in vitro* grown banana plantlets, ultimately increasing the yield.

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1.

2.

3.

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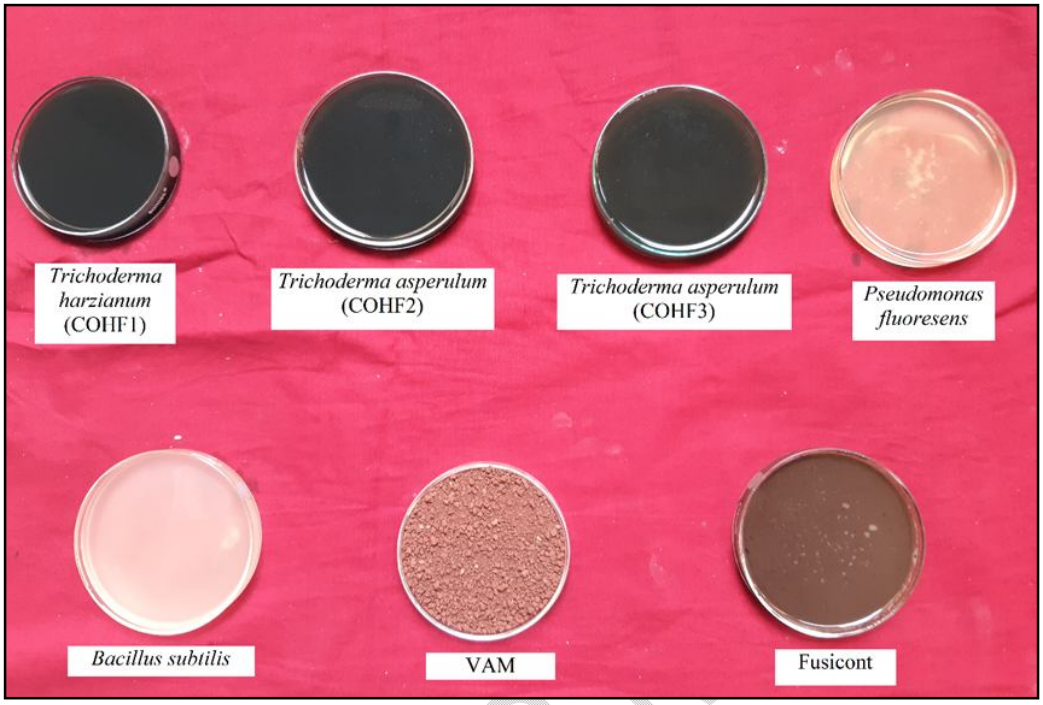
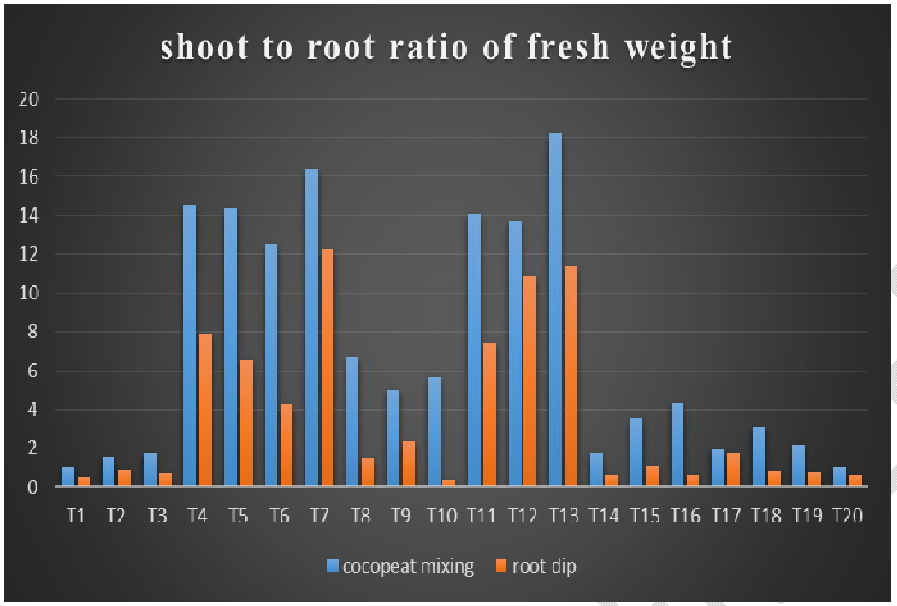
a: Powder formulation of bioinoculants



b: Liquid formulation of bioinoculants

Fig. 1: Bioagents used for biohardening

a: NanjangudRasabale after washing from tissue culture media



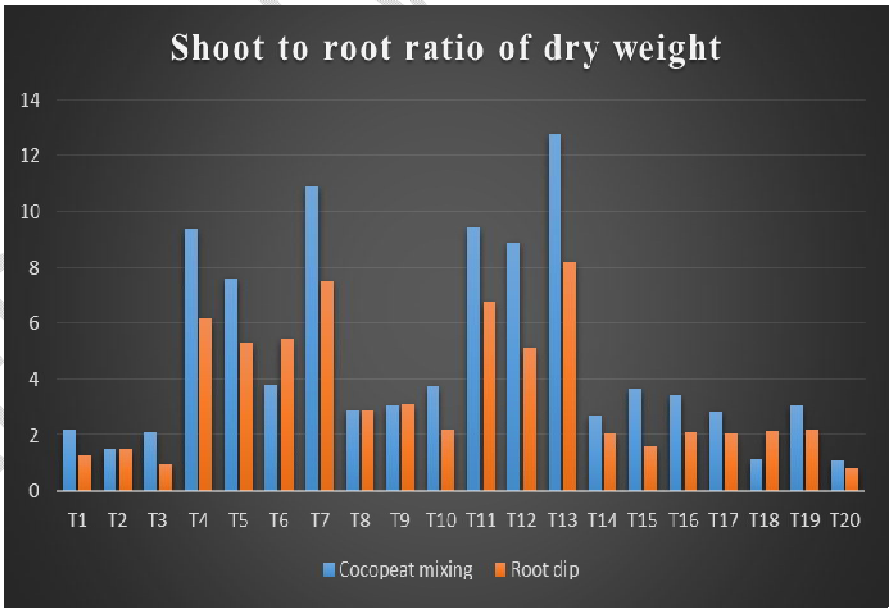


Fig. 3: Shoot to root ratio of dry / fresh weight of NanjangudRasabale plantlets after primary hardening as influenced by method of bioinoculants applications.

Table 1. Per cent plantlet survival, photosynthetic rate (SPAD units) of *in vitro* raised banana cv. NanjangudRasabale after primary hardening using different methods of bio-inoculation.

Treatment	*Ex-vitro* plantlet survival (%)			Photosynthetic rate (SPAD units)		
	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean
T_1	9.60	7.60	8.60	26.70	25.08	25.89
T_2	9.20	8.00	8.60	28.29	23.22	25.76
T_3	8.40	2.40	5.80	26.04	22.28	24.16
T_4	39.20	23.49	31.34	38.19	28.95	33.57
T_5	41.60	25.20	33.40	36.01	34.32	35.17
T_6	13.20	12.00	12.60	37.93	31.27	34.60
T_7	50.40	30.25	40.33	39.18	30.17	34.68
T_8	19.20	15.60	17.40	34.81	27.35	31.08
T_9	11.20	8.80	10.00	33.07	26.45	29.76
T_{10}	14.40	7.20	10.80	33.24	26.07	29.66
T_{11}	41.20	7.20	24.20	36.93	31.53	34.23
T_{12}	38.74	8.00	23.37	38.19	27.26	32.72
T_{13}	58.80	41.20	50.00	40.07	34.97	37.52
T_{14}	23.60	2.80	13.20	32.10	22.60	27.35
T_{15}	13.20	3.20	7.80	37.05	24.96	31.01
T_{16}	26.40	3.20	14.80	37.42	18.81	28.12
T_{17}	11.60	8.40	10.00	36.67	29.68	33.17
T_{18}	11.00	8.20	9.60	35.93	25.78	30.86
T_{19}	12.20	14.80	13.50	36.09	26.97	31.53
T_{20}	7.80	2.38	5.09	24.74	18.72	21.73
Mean	23.05	12.00		34.43	26.82	
LSD $_{(p=0.05)}$	S.Em±	$LSD_{(p=0.05)}$	CD 1%	S.Em±	$LSD_{(p=0.05)}$	CD 1%
Method (M)	0.21	0.76	1.47	0.13	0.47	0.94
Bio-agent (B)	0.80	2.97	4.66	0.50	1.83	2.99
M x B	1.61	5.94	6.59	0.99	3.65	4.24

T_1 - *Trichoderma harzianum*(COHF1), **T_2** – *T. asperullum*(COHF2), **T_3** – *T. asperullum*(COHF3), **T_4** - *Pseudomonas fluorescens*,**T_5** - *Bacillus subtilis*, **T_6** – AMF, **T_7** – Fusicont, **T_8** – AMF + *T. harzianum* (COHF1), **T_9** – AMF + *T. asperullum* (COHF2), **T_{10}** – AMF + *T. asperullum*

(COHF3), **T_{11} –** AMF + *P. fluorescens*,**T_{12} –** AMF + *B. subtilis*,**T_{13} –** AMF + Fusicont, **T_{14} –** AMF + *T. harzianum* (COHF1) + *P. fluorescens*,**T_{15} –** AMF + *T. harzianum* (COHF1) + *B. subtilis*,**T_{16} –** AMF + *T. asperullum* (COHF2) + *P. fluorescens*,**T_{17} –** AMF + *T. asperullum* (COHF2) + *B. subtilis*,**T_{18} -** AM+ *T. Asperullum* (COHF3) + *P. fluorescens*,**T_{19} –**AMF **+** *T. Asperullum* (COHF3) + *B. subtilis*,**T_{20} -** Untreated control and **CP-** Cocopeat

Table 2. Pseudostem height, pseudostem girth, number of leaves/ plantlets and leaf area (cm^2) in banana plantlets of cv. NanjangudRasabale after primary hardening as influenced by different methods of bioinoculant application.

Treatment	Pseudostem height (cm)			Pseudostem girth (cm)			No.of leaves/ plantlet			Leaf area (cm^2)		
	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean
T_1	1.58	1.40	1.49	1.48	0.74	1.11	1.85	1.67	1.76	7.29	4.87	6.08
T_2	1.53	1.41	1.47	1.63	0.96	1.29	1.91	1.53	1.72	7.70	5.63	6.67
T_3	1.93	1.11	1.52	1.35	0.13	0.74	2.60	1.13	1.87	8.57	2.33	5.45
T_4	4.87	2.15	3.51	4.01	2.74	3.38	3.75	2.40	3.07	22.91	11.28	17.09
T_5	3.80	2.36	3.08	3.83	2.61	3.22	2.93	2.11	2.52	17.78	11.15	14.46
T_6	2.86	1.86	2.36	3.31	1.98	2.65	3.00	1.73	2.37	15.95	9.25	12.60
T_7	5.53	3.21	4.37	4.24	3.62	3.93	3.93	3.07	3.50	27.76	12.85	20.31
T_8	3.06	1.86	2.46	1.44	0.20	0.82	2.80	1.87	2.33	10.43	5.68	8.06
T_9	2.92	1.71	2.31	2.37	1.26	1.81	2.40	1.87	2.13	6.75	3.93	5.34
T_{10}	2.85	1.90	2.38	2.07	1.51	1.79	2.67	1.36	2.01	13.26	8.06	10.66
T_{11}	4.46	1.89	3.18	3.89	2.39	3.14	3.20	2.40	2.80	15.45	10.01	12.73
T_{12}	3.85	2.07	2.96	4.06	1.23	2.65	3.24	2.33	2.78	17.38	11.12	14.25
T_{13}	6.46	3.63	5.05	5.42	3.51	4.46	4.07	3.33	3.70	33.15	17.03	25.09
T_{14}	2.67	1.20	1.94	2.80	0.20	1.50	2.53	1.01	1.77	13.50	1.67	7.58
T_{15}	2.99	1.00	2.00	2.37	0.06	1.21	2.67	1.02	1.84	13.16	1.87	7.51
T_{16}	2.24	0.90	1.57	3.79	0.18	1.99	2.93	1.16	2.05	11.96	1.90	6.93
T_{17}	2.41	1.96	2.18	1.81	0.06	0.94	2.60	1.87	2.24	12.83	7.25	10.04
T_{18}	2.05	1.87	1.96	1.48	2.61	2.04	1.92	0.96	1.44	12.32	8.45	10.38
T_{19}	2.33	1.85	2.09	1.71	0.73	1.22	1.86	1.15	1.51	7.87	7.80	7.83
T_{20}	1.47	0.88	1.18	1.19	0.05	0.62	1.68	1.14	1.41	6.26	1.53	3.90
Mean	3.10	1.81		2.71	1.34		2.73	1.75		14.11	7.18	
LSD $_{(p=0.05)}$	**S.Em±**	**CD 5%**	**CD 1%**	**S.Em±**	**CD 5%**	**CD 1%**	**S.Em±**	**CD 5%**	**CD 1%**	**S.Em±**	**CD 5%**	**CD 1%**
Method (M)	0.05	0.18	0.24	0.02	0.06	0.25	0.04	0.15	0.19	0.11	0.42	1.75
Bio-agent (B)	0.18	0.57	0.74	0.07	0.24	0.80	0.12	0.46	0.60	0.45	1.64	5.56
M x B	0.26	0.80	1.05	0.13	0.48	1.14	0.21	0.65	0.85	0.89	3.29	7.86

T_1 - *Trichoderma harzianum*(COHF1), **T_2** – *T. asperullum*(COHF2), **T_3** – *T. asperullum*(COHF3), **T_4** - *Pseudomonas fluorescens*,**T_5** - *Bacillus subtilis*, **T_6** – AMF, **T_7** – Fusicont, **T_8** – AMF + *T. harzianum* (COHF1), **T_9** – AMF + *T. asperullum* (COHF2), **T_{10}** – AMF + *T. asperullum* (COHF3), **T_{11}** – AMF + *P. fluorescens*,**T_{12}** – AMF + *B. subtilis*,**T_{13}** – AMF + Fusicont, **T_{14}** – AMF + *T. harzianum* (COHF1) + *P.*

fluorescens,**T_{15} –** AMF + *T. harzianum* (COHF1) + *B. subtilis*,**T_{16} –** AMF + *T. asperullum* (COHF2) + *P. fluorescens*,**T_{17} –** AMF + *T. asperullum* (COHF2) + *B. subtilis*,**T_{18} -** AM+ *T. Asperullum* (COHF3) + *P. fluorescens*,**T_{19} –**AMF **+** *T. Asperullum* (COHF3) + *B. subtilis*,**T_{20} -** Untreated control and **CP-** Cocopeat

Table 3. No. of primary roots/plantlet, No. of secondary roots/plantlet and length of longest primary root (cm) of banana plantlets of cv. NanjangudRasabale after primary hardening as influenced by method of bioinoculant applications.

Treatment	No. of primary roots/ plantlet			No. of secondary roots/ plantlet			Length of longest primary root (cm)		
	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean	CP mixing	Root dip	Mean
T_1	2.68	1.68	2.18	10.80	8.20	9.50	9.69	5.53	7.61
T_2	2.22	1.32	1.77	9.82	8.93	9.38	9.66	4.58	7.12
T_3	2.41	1.01	1.71	10.00	2.47	6.23	10.65	1.43	6.04
T_4	5.53	3.07	4.30	17.73	9.87	13.80	20.56	9.22	14.89
T_5	5.40	3.20	4.30	20.13	9.00	14.57	19.72	6.76	13.24
T_6	5.20	2.80	4.00	16.73	9.63	13.18	19.79	5.17	12.48
T_7	6.07	4.00	5.03	21.93	11.07	16.50	27.87	11.18	19.52
T_8	3.87	1.27	2.57	12.27	2.33	7.30	15.81	6.76	11.29
T_9	3.47	2.00	2.73	12.14	4.07	8.11	14.38	6.01	10.20
T_{10}	2.86	2.13	2.50	12.69	5.24	8.96	11.89	5.21	8.55
T_{11}	4.47	3.07	3.77	16.07	9.33	12.70	17.43	6.76	12.09
T_{12}	4.27	3.33	3.80	16.53	9.27	12.90	23.29	10.77	17.03
T_{13}	6.40	4.73	5.57	18.87	12.13	15.50	27.38	10.01	18.69
T_{14}	3.56	1.95	2.75	14.26	3.22	8.74	16.47	2.33	9.40
T_{15}	3.35	2.31	2.83	12.60	3.30	7.95	14.98	1.80	8.39
T_{16}	3.82	1.89	2.86	12.87	2.93	7.90	15.76	1.73	8.75
T_{17}	3.62	2.20	2.91	11.80	2.53	7.17	15.25	4.95	10.10
T_{18}	3.80	1.45	2.63	12.13	2.33	7.23	10.35	7.16	8.76
T_{19}	3.67	2.40	3.03	13.60	2.69	8.14	8.04	8.81	8.43
T_{20}	1.95	1.02	1.72	9.15	2.30	6.15	7.78	1.41	6.01
Mean	3.98	2.41		14.11	5.99		15.84	5.87	
LSD $_{(p=0.05)}$	**S.Em±**	**CD 5%**	**CD 1%**	**S.Em±**	**CD 5%**	**CD 1%**	**S.Em±**	**CD 5%**	**CD 1%**
Method (M)	0.01	0.01	0.25	0.04	0.15	0.66	0.06	0.24	0.78
Bio-agent (B)	0.01	0.05	0.79	0.16	0.59	2.10	0.25	0.93	2.47
M x B	0.03	0.11	1.12	0.32	1.17	2.98	0.51	1.87	3.49

T_1 - *Trichoderma harzianum*(COHF1), **T_2** – *T. asperullum*(COHF2), **T_3** – *T. asperullum*(COHF3), **T_4** - *Pseudomonas fluorescens*,**T_5** - *Bacillus subtilis*, **T_6** – AMF, **T_7** – Fusicont, **T_8** – AMF + *T. harzianum* (COHF1), **T_9** – AMF + *T. asperullum* (COHF2), **T_{10}** – AMF + *T. asperullum* (COHF3), **T_{11}** – AMF + *P. fluorescens*,**T_{12}** – AMF + *B. subtilis*,**T_{13}** – AMF + Fusicont, **T_{14}** – AMF + *T. harzianum* (COHF1) + *P. fluorescens*,**T_{15}** – AMF + *T. harzianum* (COHF1) + *B. subtilis*,**T_{16}** – AMF + *T. asperullum* (COHF2) + *P. fluorescens*,**T_{17}** – AMF + *T. asperullum* (COHF2) + *B. subtilis*,**T_{18}** - AM+ *T. Asperullum* (COHF3) + *P. fluorescens*,**T_{19}** –AMF + *T. Asperullum* (COHF3) + *B. subtilis*,**T_{20}** - Untreated control and **CP**- Cocopeat