Comparative Three Years Field Study of Planting Material Generated Through Tissue Culture and Conventional Fasciculated Root of Safed Musli

ABSTRACT

Aims: The present studied was aimed to investigate comparison between conventional and tissue culture raised plantlets of safedmusli at field condition.
Study design: The experiment was laid out in completely randomized block design with 8 repetitions.
Place and Duration of Study: The experiment was conducted during the *kharif* season for three consecutive years (2018-19, 2019-20 and 2020-21) at Medicinal and Aromatic Plants Research Station, Anand Agricultural University, Anand, Gujarat.
Methodology: Uniform sizes of single sprouted fasciculated root and 30-35 days old secondary hardened plantletswere used as conventional plantingmaterials and tissue culture raised plantlets respectively. Morpho-physiological parameters at 30, 60 and 90 days after transplanting and yield and quality attributing parameters were recorded after harvesting.
Results: The analysis of variance exhibited that significant difference among the planting materials during the individual years in most of the parameters. Conventional planting materials produced more vigorous growth behaviour in terms of leaves per plant, chlorophyll content and leaf area than tissue culture raised. Maximum number of fasciculated root per plant (13.72), length (9.43 cm), girth (2.70 cm), fresh (21.31 g) and dry (3.03 g) weight with greater dry recovery rate (14.99 %) and saponin content (2.16 %) was exhibited in conventional planting materials. The same planting materials were also registered higher survival rate (80.59 %) at field condition than tissue culture raised (59.38 %) planting material.
Conclusion: Therefore, it is suggested that conventional plating materials to be choose for safedmusli cultivation in *kharif* season for greater survival and higher yield. There is a need to be strengthening the present *in vitro* protocol and acclimatization process of safedmusli.

Keywords: Safed musli, fasciculated root, tissue culture, growth, survival

1. INTRODUCTION

Safed Musli (*Chlorophytum borivilianum* Sant & Fern) is wonderful endangered medicinal plant (Desale, 2013) native to India and considered as a 'white gold' or 'divyaaushad' in Indian systems of medicine (Khanam *et al*., 2013). This endangered species is distributed mostly in Assam, Eastern Ghats, Eastern Himalayas, Bihar and Andra Pradesh in India (Garima and Shruti, 2012); it is also reported that the crop is grown naturally in hilly areas of Gujarat, Rajasthan and Madhya Pradesh. Safed musli belonging to the family *Liliaceae* and main economic part of the crop is fasciculated root (tuber).

It is a rich source of over 25 alkaloids, vitamins, minerals, proteins, carbohydrates, steroids-saponins and polysachharides*etc*. The major contents of safedmusli are carbohydrate (42%), proteins (8-9%), root fiber (3-4%) and saponin (2.17%) (Desale, 2013). It is reported that safedmsuli use enhances vitality and immunity. Besides that, it has antimicrobial, anti-

inflammatory and antitumor properties (Thakur *et al*., 2009); it was also revealed that it is used in arthritis, diabetes, rheumatism and joint pain (Acharya *et al*., 2009). Annual demand of safedmusli is estimated to be about 35,000 MT in India but we are producing and collecting very less quantity, 5,000 MT per year. Even at the international market, demand of safedmusli dried powder is increasing. We all know that most of the medicinal plants have been collected from the wild condition and now the natural sources are depicted speedily. Fasciculated roots (tubers) are commonly used as propagation materials. Each fasciculated roots fingers are separated in such a way that each finger has a portion of crown disk attached to it for the better sprouting and survival when it transfers to field. Seeds are used less often for planting due to their poor germination, low viability and long dormancy period (Jat and Bordia, 1990). Therefore, it requires large quantity of planting materials about 80,000 fingers weighing 10-12 quintals for one-hectare planting in a distance of 35 x 10 cm. The crop is normally harvested in October month (120-125 DAS) for the purpose of raw material use (*i.e*., drug). However, for the planting material purpose fleshy fasciculated root bunches are allowed to remains in soil. Then aerial parts are completely dry and fall down on soil surface in the month of Jan-Feb and finally, roots are ready to harvest in March (Anon. 2015).

Moreover, *in vitro* micro-propagated plantlet is also used for their rapid, uniform and disease-free planting materials production. But survival of *in vitro* plantlets in *ex vitro* conditions is an issue exhibited in micro propagation techniques. The best and success *in vitro*technique is considered when it relies to endure at filed conditions (Shekhawat and Manokari, 2018). Earlier it was reported that low survival rate of micro propagated plantlets at filed conditions in various crops like *Dolichandra unguis-cati* (Soni *et al*., 2021), rhododendron (Valero-Aracama*et al*., 2001), sugarcane (Tolera and Shimelis, 2016) and as well as low yield in plantain (Vuylsteke and Ortiz, 1996) and banana (Israeli *et al*., 1988). However in vitro plant performance under field conditions in terms of quantity and quality are well established in many crops. On this context, the present investigation was framed and executed to evaluate the growth and development, quality and survival rate of safedmusli planting materials generated from conventional fasciculated root and tissue culture raised plantlets.

2. MATERIAL AND METHODS

2.1 Site description

The experiment was conducted during the *kharif* season for three consecutive years (2018-19, 2019-20 and 2020-21) at Medicinal and Aromatic Plants Research Station, Anand Agricultural University, Anand, Gujarat.The climatic conditions during the crop growth period of three consecutive experimental years was presented in terms of Meteorological Standard Week (MSW) in Figure 1 (July to October).The texture of the soil of the experimental plot has loamy sand type with very deep and fairly moisture holding capacity.

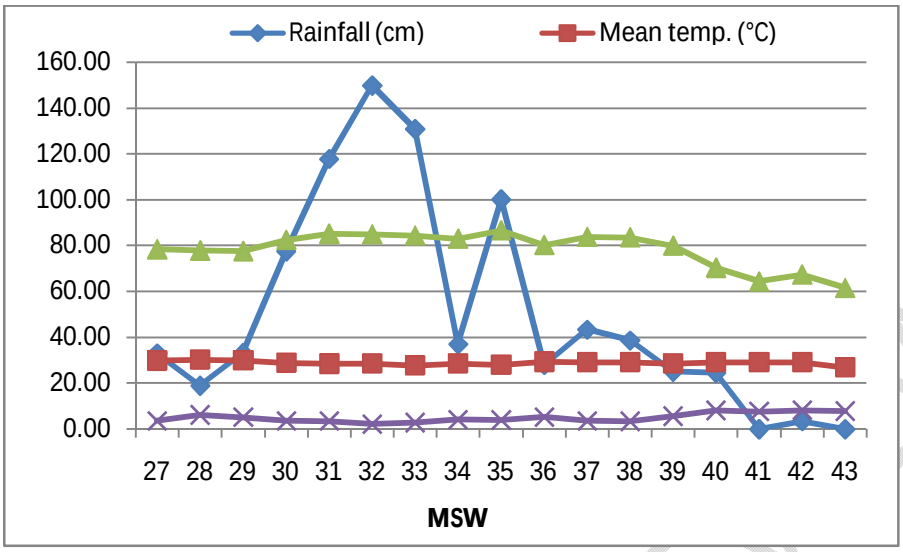

Figure 1: Three years mean climatic condition of the experimental site from July to October

2.2 Planting materials and experimental design

The planting materials used in the experiment comprised of conventional planting materials (CPM) *i.e.*fasciculated root and tissue culture raised plantets (TCRP) (Figure 2). Uniform sizes of single sprouted fasciculated root were selected as CPM. Meanwhile, 30-35 days old secondary hardened tissue culture raised plantlets were used in the experiment. All the planting materials were planted with the spacing of 30 x 10 cm during 1st fortnight of July in all the experimental years and it was harvested in 1st fortnight of March (240-250 DATP). Normal agronomical practices were followed during the crop growth period.The experiment was laid out in completely randomized block design with 8 repetitions.

Fasciculated root | Tissue culture raised plantlet

Figure 2: Planting materials used in the experiment

2.3 Morpho-physiological parameters

Morpho-physiological data were collected at 30, 60 and 90 DATP which included plant height (PH; cm), leaves per plants (LPP), leaf width (LW; cm) chlorophyll content (CC; SPAD value) and leaf area (LA; cm^2). The above morpho-physiological parameters were recorded on five competitive plants per plots on each treatment (planting materials) and average value was used in statistical analysis. The PH was measured from the soil surface to topmost tip of leaf and LW at the mid portion of the leaf. Similarly, chlorophyll content was measured using chlorophyll meter (SPAD-502 Plus, Konica Minolta, INC, Japan). Leaf area was evaluated using CID Bio-Science based leaf area meter (Model No. CI-203) and expressed in cm^2.

2.4 Yield and quality related parameters

The observed yield related parameter comprised of fasciculated root per plant (FRPP), fasciculated root length (FRL; cm) and girth (FRG; cm), fasciculated root fresh (FRFW; g) and dry weight(FRDW; mg) and dry recovery (%). The quality parameters included saponin (%), starch (%) andfibre content (%). Moreover, number of plants stand (survival rate) was observed at the time of harvesting. For the quality analysis aspects, peel was removed from the cleaned harvested fasciculated roots using the sharp knife and dried under the sun light condition. Finally make the powder and used in downstream analysis (Figure 3).

Fresh fasciculated root | Peeled root | Dried root | Powder

Figure 3: Powder preparation from freshly harvested fasciculated root

Fibre content (FC) was analysed through acid–base hydrolysis method (AOCC, 2002) and calculated using the given equation.

$$FC\ (\%) = \frac{(\text{Wt. of residue} - \text{Wt. of ash})}{\text{Wt. of sample}} x100$$

The saponin content was analysed through slightly modification of protocol developed by Sim, 2011. To prepare standard curve, 20mg saponin powder (Sigma Aldrich) was dissolved in 100mL distilled water and an aliquot of 0.2, 0.4, 0.6, 0.8 and 1.0mL of standard solution was used in standardization of standard curve. 0.5g of dried sample powder was dissolved in 50mL aqueous methanol (85%). The solution was shaken well by using a mechanical shaker (Rotek-LSV, Pelican Equipments) for 30 min. and followed by filtered using a whatman filter paper 1 and the process was repeated thrice. The extract was collected and evaporated to dryness on water bath (NB-5, Nuve). Residue was dissolved in 50mL distilled water in a Volumatic flask. Finally, 100mL volume was made by dissolving 10mL of diluted solution with distilled water. Followed by 0.2mL of sample solution was mixed with 0.8mL distilled water and 5mL of freshly prepared vanillin sulphuric acid reagent. The said reagent was prepared freshly by dissolving 0.7g vanillin with 100mL of 65% conc. H_2SO_4 in chilled condition. Further, the mixture was put in water bath at 60ºC for 1 hour and tubes were cool in ice-cold water bath for 4-5 mins. Absorbance was read at 430nm against the blank using the spectrophotometer (SL-218, Elico). Anthronesulphuric acid method was used for starch analysis by taking absorbance at 630nm (Hansen and Moller, 1975).

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance (ANOVA)

ANOVA exposed a significant difference ($p < 0.05$) among the planting materials for all studied in individual environments and across environments with respect to the morpho-physiological, yield related and other quality parameters (Table 1 and 2). This suggested the

presence of adaptability variation in the studies planting materials for the traits. The year mean squares were also significant ($p < 0.05$) for all the parameters this strongly significant that environmental conditions play pivotal role in crop adaptability, growth and development ultimately it effects on quality parameters. Among the planting materials based on pooled mean sum of squares was found non-significant in all the parameters except LW at 60 and 90 DATP, CC at 30 and 90 DATP, FRL, FRFW and survival rate. Interaction of year into planting materials (Y × P) showed significant data based on pooled analysis in all the morpho-physiological and yield and other quality related parameters except in LW 60 and 90 DATP and CC at 90 DATP.

Table 1: Mean squares of various morpho-physiological parameters of safedmusli

Source of variation		Year (Y)	Planting materials (P)	Y x P	Error
DF#		2	1	-	14
PH at 30 DATP	Y1	-	254.32*	-	2.65
	Y2	-	1.27NS	-	1.67
	Y3	-	430.67*	-	1.14
	Pooled	327.86*	476.91NS	104.67*	1.82
PH at 60 DATP	Y1	-	0.001NS	-	3.34
	Y2	-	9.63*	-	2.05
	Y3	-	93.07*	-	1.47
	Pooled	203.63*	54.46NS	24.12*	2.29
PH at 90 DATP	Y1	-	33.15*	-	2.99
	Y2	-	23.52*	-	2.95
	Y3	-	57.42*	-	1.84
	Pooled	8.64*	14.83NS	49.63*	2.60
LW at 30 DATP	Y1	-	19.94*	-	0.32
	Y2	-	1.40NS	-	0.31
	Y3	-	45.73*	-	0.29
	Pooled	34.68*	51.36NS	7.86*	0.31
LW at 60 DATP	Y1	-	25.25*		0.42
	Y2	-	40.01*		0.39
	Y3	-	29.54*		0.56
	Pooled	0.44*	93.91*	0.44NS	0.46
LW at 90 DATP	Y1	-	20.30*		0.44
	Y2	-	10.79*		0.49
	Y3	-	10.79		0.29
	Pooled	0.01*	40.89*	0.50NS	0.41
LPP at 30 DATP	Y1	-	43.36*	-	0.48
	Y2	-	318.09*	-	1.98
	Y3	-	152.09*	-	0.82
	Pooled	664.26*	450.25NS	31.65*	1.09
LPP at 60 DATP	Y1	-	997.61*	-	2.36
	Y2	-	122.88*	-	2.49
	Y3	-	855.56*	-	3.09
	Pooled	202.39*	1724.39NS	125.95*	2.65
LPP at	Y1	-	8.51*	-	0.27

90	Y2	-	45.56*	-	0.97
DATP	Y3	-	152.09*	-	2.19
	Pooled	934.67*	161.33NS	22.42*	1.14
CC at	Y1	-	170.37*	-	2.42
30	Y2	-	289.59*	-	2.25
DATP	Y3	-	430.36*	-	1.26
	Pooled	88.04*	860.72*	14.80*	1.98
CC at	Y1	-	19.54*	-	3.61
60	Y2	-	12.39*	-	2.08
DATP	Y3	-	64.12*	-	1.76
	Pooled	200.76*	16.84NS	39.60*	2.48
CC at	Y1	-	4.44NS	-	1.34
90	Y2	-	13.62*	-	0.99
DATP	Y3	-	3.44NS	-	1.51
	Pooled	6.21*	19.52*	0.99NS	1.28
LA at 30	Y1	-	25.88*	-	1.14
DATP	Y2	-	231.12*	-	1.85
	Y3	-	173.84*	-	1.47
	Pooled	164.33*	373.53NS	28.66*	1.49
LA at 60	Y1	-	50.66*	-	2.86
DATP	Y2	-	1.45NS	-	2.93
	Y3	-	3.36NS	-	4.08
	Pooled	92.68*	14.03NS	20.72*	3.29
LA at 90	Y1	-	15.64*	-	2.37
DATP	Y2	-	2.92NS	-	1.47
	Y3	-	5.18NS	-	3.00
	Pooled	92.87*	3.83NS	9.95*	2.28

#PH: Plant height, LPP: leaves per plant, LW: Leaf width, CC: Chlorophyll content, LA: Leaf area, Y1: 2028-19, Y2: 2019-20, Y3: 2020-21, * Significant, NS: Non-significant

Table 2: Mean squares of various yield related and quality parameters of safedmusli

Source of variation	Year (Y)	Planting materials (P)	Y x P	Error
DF#	2	1	-	14
FRPP Y1	-	101.00*	-	0.92
Y2	-	113.69*	-	0.45
Y3	-	18.28*	-	0.52
Pooled	71.86*	208.13NS	12.42*	0.63
FRL Y1		7.59*		0.23
Y2		12.06*		0.23
Y3		12.30*		0.19
Pooled	7.41*	31.59*	0.18NS	0.22
FRG Y1		0.06NS		0.02
Y2		3.18*		0.01
Y3		1.77*		0.01
Pooled	1.95*	2.73NS	1.14*	0.02

FRFW	Y1		477.75*		1.21
	Y2		588.67*		0.47
	Y3		263.90*		0.86
	Pooled	39.17*	1296.46*	16.93*	0.85
FRDW	Y1		6.46*		0.01
	Y2		27.34*		0.04
	Y3		6.97*		0.01
	Pooled	3.27*	36.13^{NS}	2.31*	0.02
Saponin content	Y1		4.28*		0.01
	Y2		0.27*		0.02
	Y3		0.66*		0.01
	Pooled	0.95*	3.86^{NS}	0.68*	0.01
Starch content	Y1		3.87*		0.58
	Y2		0.05^{NS}		0.90
	Y3		0.57^{NS}		0.72
	Pooled	9.95*	2.89^{NS}	0.80^{NS}	0.75
Crude fibre content	Y1		0.77*		0.01
	Y2		0.02^{NS}		0.02
	Y3		0.17*		0.01
	Pooled	0.12*	0.03^{NS}	0.47*	0.01
Dry recovery	Y1		0.20^{NS}		0.47
	Y2		31.38*		1.42
	Y3		17.60*		0.51
	Pooled	272.04*	35.00^{NS}	7.09*	0.80
Survival	Y1		1799.88*		11.37
	Y2		1530.77*		13.97
	Y3		2086.21*		9.23
	Pooled	168.72*	5395.39*	10.73^{NS}	11.52

[#]FRPP: Fasciculated root per plant, FRL: Fasciculated root length, FRG: Fasciculated root girth, FRFW: Fasciculated root fresh weight, FRDW: Fasciculated root dry weight, Y1: 2018-19, Y2: 2019-20, Y3: 2020-21, * Significant, NS: Non-significant

3.2 Means performances of morpho-physiological parameters

Descriptive statistics of pooled data are presented in Table 3. Mean performances analysis suggested that differences among the planting materials for most of the traits was non-significant but it was found significant in all the individual years. Non-significant differences in pooled analysis indicated that performances of planting materials in individual years in field condition were inconsistent and it was might be due to the differed in growing environment condition during the individual year. In all the morpho-physiological parameters the maximum vegetative growth rate was observed at 60 DATP followed by decreased in growth rate. The results of mean performance based on pooled data showed that PH observed at 30, 60 and 90 DATP was found higher in CPM and it was respectively 32.36, 7.78 and 4.75 percent increase over TCRP. Maximum number of leaves per plant was revealed under the CPM *i.e.*, 16.72, 27.15 and 26.52 at 30, 60 and 90 DATP respectively. Similarly, in LW and CC, CPM showed that better leaf width development and chlorophyll pigmentation over the TCRP in all the recorded growth stages. However, in leaf area at initial growth stages (30 DATP) CPM had maximum leaf area but in later growth stages it was registered in TCRP.

Tissue culture raised plantlets had 30.41 and 22. 75cm^2 leaf area at 60 and 90 DATP respectively as compared to CPM (29.33 and 22.19cm^2).

Being a tuberous crop massive deposition of C and N assimilates such as starch and storage proteins are stored in such organ (Appeldoorn *et al*., 1999). Generally, it was observed that the remobilization of reserves food materials from the storage organs or endosperm to the active growth region by providing essential energy until the seedling becomes photoautotrophic. It is also reported that with the beginning of sprouting in potato tuber, such organ become a source for the growing sprout (Sonnewald, 2001). In safedmsuli, fasciculated root contains 35-45% carbohydrates (Goyal *et al*., 2018) along with 8-8.5% proteins (Nikam, 2017) which contributes huge amount of energy during the development of shoot and root and resulting in early and luxurious morphological growth was observed as compared to the tissue culture raised plantlets. Apart from this, it had been observed that secondary hardened tissue culture raised plants might not be able to adapt as early as requirement at filed condition in some of the field crops. Bhojwani and Dhawan, 1989 mentioned that abnormal leaf morphology and anatomy, lower photosynthesis, deformity in stomata structure and decrease in cuticular wax layer of leaves under *in vitro* plants due to the rapid growth and multiplication of shoots under *in vitro*micro-propagation techniques. Growing in very optimises environmental condition, external supplement of food and unable to control water loss in render micro-propagated plants susceptible to the transplantation shocks and its transplantation juncture becomes a major bottleneck in *in vitro* micro-propagation techniques in many plants (Conner and Thomas, 1981; Ziv, 1986). It has been well established that reduction in growth, yield and quality by water stress in field conditions in most of the crop due to the transplanting shock (Kriedeman and Barrs, 1981).

3.3 Means performances of yield related and quality attributing parameters

The data pertaining to yield related and quality parameters based on pooled analysis is depicted in Table 4. Mean performances based on pooled data showed that significant differences in yield related parameters like FRPP, FRL, FRFW, FRDW and survival rate recorded at the time of harvesting however it was non-significantly differences in FRG, saponin, starch, crude fibre and dry recovery among the planting materials. This indicated that the more suitability and better growth and development in the set of studied of planting materials studies over the environments. CPM was found maximum FRPP (44.27), FRL (9.81cm), FRG (2.70cm), FRFW (21.31g), FRDW (132.83mg) and it was 44.27, 20.74, 21.62, 95.15 and 132.82 percent increase over the TCRP respectively.

In case of quality parameters, numerically higher saponin content (2.16%) and dry recovery rate (14.29%) with maximum survival rate of 80.59% was disclosed in CPM as compared to TCRP (17.92, 12.58 and 59.38%), respectively. However, in starch (17.92%) and crude fibre content (2.44%) it was maximum in tissue culture raised plantlets.

As discussed earlier, conventional planting materials had better growth and development at field condition as compared to tissue culture raised plants in the present study. Photosynthetic apparatus is not well developed in tissue culture raised plantlets due to the heterotrophic mode of nutrition; moreover, poor chlorophyll synthesis and enzymes responsible for photosynthesis are inactive or absent in micro propagated plants (Donnelly and Vidaver, 1984). Comparatively, it was witnessed that more accumulation of photosynthate to the sink organ (new tubers) resulting in a greater number of fasciculated root with higher length and girth in conventional planting materials at present experiment. There was less plants stand (survival rate) at the time of harvest in tissue culture raised

plants. Hazarika, 2003 was also reported that micro-propagated plants do not survive well at green house and field condition. Low relative humidity, high light intensity and septic environmental conditions exhibit more stressful to micro-propagated plants as compared to the normal plants. Therefore, tissue culture raised plantlets particularly in safedmusli consistently showed inferior performance in terms of growth and development, yield and quality with higher mortality in field condition. In future well standardized tissue culture protocol of safedmusli is much necessary. In the similar way acclimation process appeared to be an important factor to overcome from transplanting shock at field condition.

4. CONCLUSION

Considering all these finding in present experiment it is concluded that conventional fasciculated root of safedmusli are more adaptable and higher survival rate at field condition. Secondary hardened tissue culture raised plantlets experienced high mortality when it's transferred to field condition. There is a great reduction in plant morpho-physiological growth parameters resulting in inferior in yield and yield attributing traits in tissue culture raised plantlets transplanted during the *kharif* season. Therefore, researchers should more focus on morphological and anatomical structural changes of the crop during *in vitro* developmental network and need to be optimised the secondary hardening process to have better survival rate and profuse growth and development particularly in this crop.

Table 3: Descriptive statistics (pooled data) of morphological traits recorded at 30, 60 and 90 DATP of safedmusli

Planting materials	PH (cm)			LPP			LW (cm)			CC (SPAD value)			LA (cm^2)		
	30 DATP	60 DATP	90 DATP	30 DATP	60 DATP	90 DATP	30 DATP	60 DATP	90 DATP	30 DATP	60 DATP	90 DATP	30 DATP	60 DATP	90 DATP
TCRP	19.50	27.38	23.38	10.60	15.17	12.85	8.18	10.93	9.05	18.75	25.15	22.30	16.57	30.41	22.75
CPM	25.81	29.51	24.49	16.72	27.15	16.52	10.25	13.73	10.90	27.22	26.34	23.57	22.15	29.33	22.19
% increased	32.36	7.78	4.75	57.74	78.97	28.56	25.31	25.62	20.44	45.17	4.73	5.70	33.68	-3.55	-2.46
S.Em.±	2.09	1.00	1.44	1.15	2.29	0.97	0.57	0.19	0.18	0.79	1.28	0.32	1.09	0.93	0.64
C.D. at 5%	NS	NS	NS	NS	NS	NS	NS	0.56	0.53	4.78	NS	NS	NS	NS	NS
Year effect	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Y x T, S.Em.±	0.48	0.53	0.57	0.37	0.58	0.38	0.20	0.24	0.23	0.50	0.56	0.40	0.43	0.64	0.53
C.D. at 5%	1.36	1.53	1.63	1.06	1.64	1.08	0.56	NS	NS	1.42	1.59	NS	1.23	1.83	1.53
C.V.%	5.96	5.32	6.73	7.65	7.69	7.28	6.03	5.47	6.41	6.12	6.12	4.93	6.31	6.07	6.72

#TCRP: Tissue culture raised plantlets; CPM: Conventional planting materials; NS-Non-significant; S: Significant

Table 4: Descriptive statistics (pooled data) of yield and yield related parameters, quality and survival rate of safedmusli

Planting materials	Fasci. root per plant	Fasci. root length (cm)	Fasci. root girth (cm)	Fasci. fresh root weight (g)	Fasci. dry root weight (mg)	Saponin content (%)	Starch content (%)	Crude Fibre content (%)	Dry recovery (%)	Survival (%)
TCRP	9.51	7.81	2.22	10.92	1.31	1.59	17.92	2.44	12.58	59.38
CPM	13.72	9.43	2.70	21.31	3.05	2.16	17.43	2.39	14.29	80.59
% increased	44.27	20.74	21.62	95.15	132.82	35.85	-2.73	-2.05	13.59	35.72
S.Em.±	0.72	0.13	0.22	0.84	0.31	0.17	0.24	0.14	0.54	0.96
C.D. at 5%	2.23	0.38	NS	5.11	0.87	NS	NS	NS	NS	2.80
Year effect	S	S	S	S	S	S	S	S	S	S
Y x T, S.Em.±	0.28	0.16	0.04	0.33	0.05	0.04	0.31	0.04	0.32	1.20
C.D. at 5%	0.80	NS	0.13	0.93	0.15	0.11	NS	0.12	0.90	NS
C.V.%	6.80	5.40	5.11	5.71	6.89	5.98	4.89	4.84	6.66	4.85

#TCRP: Tissue culture raised plantlets; CPM: Conventional planting materials; NS-Non-significant; S: Significant

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