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Effect of Different Substrates and Time of Application of Nutrient at Different Concentration on Growth Parameters of Lettuce (*Lactuca sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to determine the combined effect of different substrates and time of application of nutrient at different concentration on growth parameters of Romaine long (green) lettuce. The experiment was conducted in semi-controlled naturally ventilated polyhouse for two years (2021-2022 and 2022-2023) during *Rabi* season at Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MH). The research was laid in a Factorial Completely Randomized Design, which is replicated twice with three factors *i.e.* Factor A) consisted different substrates (S) (S_1: cocopeat, S_2: cocopeat + perlite (1:1 v/v), S_3: cocopeat +

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perlite (2:1 v/v) and S_0: soil), Factor B) levels of nutrient concentration (F) (F_1: 100%, F_2: 80% and F_3: 60%) and Factor C) application time interval (I) (I_1: one day interval, I_2: three days interval and I_3: six days interval). The nursery raising of lettuce was done under greenhouse in pro-trays and seedling are ready for transplanting one month after sowing. The result revealed that combined application of treatment T_6 [$S_2F_1I_2$] *i.e.* Cocopeat + Perlite (1:1) + 100% + Three days interval recorded maximum plant height (28.24 cm) and number of inner leaves (18.50) whereas maximum number of outer leaves per plant (5.35) was recorded in the treatment combination of T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval and T_5 [$S_1F_1I_2$] *i.e.* Cocopeat + 100% + Three days interval in pooled mean data at harvest. Further, the highest leaf area was (105.84 cm^2) was observed in the treatment combination of T_{33} [$S_1F_3I_3$] *i.e.* Cocopeat + 60% + Six days interval. The obtained result showed clear difference of lettuce growing under different substrates and time of application of nutrient at different concentration on growth parameters.

Keywords: Lettuce; cocopeat; perlite; nutrient levels; growth; grow-bags; substrate.

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a herbaceous, self pollinated annual vegetable belonging to the daisy family called Compositae and subfamily Chicoridae. It is believed to be originated in the Mediterranean region, Central Asia and South-west Asia. It is cool season vegetable which thrives in temperature ranging from 7 to 25 °C and most important salad crop generally cultivated for it's tender leaves and head. It is universal use in kind of foods, such as burger, soup, sandwiches wraps and similar food items. It is eaten as raw or cooked, widely used in chinese cookery. In India, salad crops are not grown on a commercial scale, they are mostly around the big cities and in the kitchen garden as salad crops are now being valuable because of their nutritional value in the regular diet.

In India, it is gaining popularity with the change in food habit and health increasing consciousness among the people. There is an increasing demand by consumers for safe and nutritious foods that improves the physical performance, reduces the risks of diseases and increases the life span (Ogden et al*.,* 2007). It has a high content of phytonutrients combined with a low content of dietary fats, which makes lettuce an attractive low- calorie food, whose consumption is highly suggested within weight-loss dietary plans (Kim et al. 2016). It is also known as an anodyne, sedative, diuretic and expectorant (Kallo, 1986).

Growing medium which is termed as "substrate" is defined as any solid material (alone or in mixtures) excluding soil, which guarantee better plant growth conditions than agricultural soil in one or many aspects (Gruda et al*.,* 2013). Substrates such as perlite, cocopeat, sawdust, vermicompost etc. which are less expensive and has been used as soilless substrate culture around the world for successful vegetable production. Various kinds of substrates have different function such as water holding capacity, nutrient capacity, root growth and aeration. Substrate culture under protective agriculture has minimized the discharge of fertilizer and pesticide residues into the natural environment. Many research studies reported that commercial vegetable production under controlled condition with substrates adopted to reduce economic losses caused by soil-borne pathogens. On the other hand, for an effective nutritional management and consequently, an increase in substrate lettuce yield, it is indispensable the appropriate control of nutrient solution. Among the factors to be controlled are pH, temperature, electrical conductivity and oxygen concentration, as well as the period of application time interval and concentration of nutrient solution to the plant root during crop life cycle. Different substrates have several materials which could have direct and indirect effects on plant growth and development. The primary aim of this study is to assess the growth of romaine lettuce crop, when grown in different substrate with proper supply of plant nutrient at appropriate dose and time with sufficient irrigation.

2. MATERIALS AND METHODS

The present investigations were conducted in semi-controlled naturally ventilated polyhouse for two years (2021-2022 and 2022-2023) during *Rabi* season at Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MH). The research was laid in a Factorial Completely Randomized Design (FCRD), which is replicated twice with three

factors *i.e.* Factor A) consisted different substrates (S) (S_1: cocopeat, S_2: cocopeat + perlite (1:1 v/v), S_3: cocopeat + perlite (2:1 v/v) and S_0: soil), Factor B) levels of nutrient concentration (F) (F_1: 100%, F_2: 80% and F_3: 60%) through 19:19:19 NPK and calcium nitrate and Factor C) application time interval (I) (I_1: one day interval, I_2: three days interval and I_3: six days interval).The nursery raising of "Romaine long" (green) lettuce variety seeds was done under greenhouse in pro-trays and seedling are ready for transplanting one month after sowing. Seedlings were transplanted under grow-bags having size of 12 × 16 inch filled with different substrates and soil for two growing years on date 20 to 22 November, inside the naturally ventilated polyhouse. Immediate after transplanting of lettuce seedling in grow-bags a light irrigation was given to maintain optimum soil moisture for growth. Trickle irrigation system was installed for application of water as per recommended schedule.

Commercial formulations of 4% formaldehyde solution was used to sterilize the soil and 4% calcium nitrate used to sterilize the cocopeat and perlite substrate. After that EC and pH of the substrates was checked and balanced before filling grow-bags. Recommended dose Nitrogen, phosphorus and potash were used for lettuce: 80 kg N/ha, 50 kg P_2O_5/ha and 50 kg K_2O/ha through 19:19:19:, 37.5% N calcium nitrate, magnesium sulphate and micronutrient mixture. Different concentration of nutrient solution were prepared 100%, 80% and 60% according to treatments details and this concentration was given to lettuce according to application time interval with the help of hand to each treatment 50 ml solution each time to one grow-bag. Preventive measures were taken to keep the lettuce crop pest and disease free. All the recommended package of practices was followed.

Lettuce was harvested 48 days after transplanting when they were attained good size, still young with tenderness in leaves. Five plants were randomly selected from each of 36 treatments and were labeled. The observation on growth parameters of lettuce *i.e.* plant height, number of inner leaves, number of outer leaves and leaf area were recorded at 25 days after transplanting and at the time of harvesting and subjected for statistical analysis. The results data obtained were analysed using standard statistical procedure given by Gomez and Gomez (1984).

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm)

3.1.1 Effect on plant height at 25 DAT

Among interactions, significantly the maximum plant height [(19.83), (19.91) and (19.87)] was recorded in treatment T_9 [$S_1F_1I_3$] *i.e.* Cocopeat + 100% + Six days interval and minimum [(13.33), (13.26) and (13.29)] was recorded in treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval during the year 2021-22, 2022-23 and pooled mean, respectively.

3.1.2 Effect on plant height at harvest

Among the treatment combinations, T_6 [$S_2F_1I_2$] *i.e.* Cocopeat + Perlite (1:1) + 100% + Three days interval was observed significantly the highest plant height [(28.35) and (28.24)] and lowest [(19.35) and (18.13)] was found in treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval during the year 2021-22 and pooled mean, respectively. Whereas in the year 2022-23, the maximum plant height (29.67) was recorded in the treatment combination of T_9 [$S_1F_1I_3$] *i.e.* Cocopeat + 100% + Six days interval which was followed by treatment combination of T_6 [$S_2F_1I_2$] (28.14) and minimum (16.92) was recorded in treatment combination of T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval.

The above results revealed that the plant height significantly varied due to combined effect of substrates, levels of nutrient concentration and application time interval at 25 DAT and at harvest given in Table 1 and represented in Fig. 1 during the year 2021-22, 2022-23 and pooled mean. The different substrates induced significantly positive impact on plant height, especially using of substrates S_1 compared with other substrate or the control in both the season as well as in pooled mean data found highest plant height at 25 DAT while, using of substrates S_2 compared with other substrate or the control in both the season as well as pooled mean data recorded maximum enhancement of plant height at harvest.

The plant height at different stages of growth was observed to be higher with higher dose of fertilizers. An increased in plant height at 100% nutrient concentration in grow-bags containing substrate cocopeat at six days interval at 25 DAT in pooled mean (19.87 cm) or in grow bag containing mixture of substrates cocopeat +

perlite (1:1 v/v) three days interval at harvest in pooled mean (28.24 cm) might be due to maximum uptake of nutrients resulted from better availability of sufficient quantity of major nutrients in substrates. The enhanced plant growth might be due to the fact that nitrogen with synthesized carbohydrates was metabolized into amino acids and proteins which allowed the plants to grow faster. As nitrogen, phosphorous and potassium is one of major plant nutrient required for the growth consequently its uptake increases the cell number and size leading to better growth. These result are in conformity with Sanchita et al. (2004) in broccoli, Shinde et al. (2006) in cabbage, Singh et al. (2006) in broccoli, Tanpure et al. (2007) in cabbage, Farag et al. (2013) in lettuce, Mohamed (2018) in areca palm and Nikzad et al. (2020) in cabbage.

Table 1. Interaction effect of different substrates, levels of nutrient concentration and application time interval on plant height of lettuce

Treatment No.	Treatments	Plant height (cm)					
		2021-22		2022-23		Pooled mean	
		25 DAT	Harvest	25 DAT	Harvest	25 DAT	Harvest
	Interaction: S × F × I						
T_1	$S_1F_1I_1$	18.15	25.27	17.93	26.08	18.04	25.67
T_2	$S_2F_1I_1$	16.61	25.89	16.16	24.92	16.38	25.40
T_3	$S_3F_1I_1$	19.18	27.76	18.94	28.09	19.06	27.92
T_4	$S_0F_1I_1$	13.74	22.47	13.90	22.40	13.82	22.43
T_5	$S_1F_1I_2$	17.36	24.20	17.10	24.44	17.23	24.32
T_6	$S_2F_1I_2$	17.02	28.35	17.31	28.14	17.16	28.24
T_7	$S_3F_1I_2$	14.91	24.76	14.94	24.67	14.92	24.71
T_8	$S_0F_1I_2$	18.23	26.98	17.83	25.54	18.03	26.26
T_9	$S_1F_1I_3$	19.83	26.67	19.91	29.67	19.87	28.17
T_{10}	$S_2F_1I_3$	14.67	23.55	14.31	22.74	14.49	23.14
T_{11}	$S_3F_1I_3$	16.94	26.21	17.32	24.92	17.13	25.56
T_{12}	$S_0F_1I_3$	15.94	23.92	16.19	23.58	16.06	23.75
T_{13}	$S_1F_2I_1$	15.75	20.14	15.60	22.36	15.67	21.75
T_{14}	$S_2F_2I_1$	17.51	27.35	17.70	27.96	17.60	27.65
T_{15}	$S_3F_2I_1$	16.59	24.30	16.66	23.90	16.62	24.10
T_{16}	$S_0F_2I_1$	17.48	26.31	17.70	24.43	17.59	25.37
T_{17}	$S_1F_2I_2$	17.79	26.38	17.46	27.64	17.62	27.01
T_{18}	$S_2F_2I_2$	16.20	27.08	16.31	27.54	16.25	27.31
T_{19}	$S_3F_2I_2$	18.18	26.91	18.82	23.77	18.50	25.34
T_{20}	$S_0F_2I_2$	13.43	20.95	14.33	19.29	13.88	20.12
T_{21}	$S_1F_2I_3$	16.81	23.36	16.45	24.66	16.63	24.01
T_{22}	$S_2F_2I_3$	17.58	27.32	18.82	26.57	18.20	26.94
T_{23}	$S_3F_2I_3$	14.53	22.69	14.59	20.25	14.56	21.47
T_{24}	$S_0F_2I_3$	17.26	25.45	17.34	25.91	17.30	25.68
T_{25}	$S_1F_3I_1$	18.35	27.39	18.52	27.10	18.43	27.24
T_{26}	$S_2F_3I_1$	14.90	23.12	15.40	22.78	15.15	22.95
T_{27}	$S_3F_3I_1$	18.67	25.50	17.84	26.05	18.25	25.77
T_{28}	$S_0F_3I_1$	16.20	24.61	15.99	22.62	16.09	23.61
T_{29}	$S_1F_3I_2$	15.17	22.84	15.22	23.58	15.18	23.21
T_{30}	$S_2F_3I_2$	17.55	28.01	18.42	27.58	17.98	27.79
T_{31}	$S_3F_3I_2$	16.59	25.55	16.77	26.04	16.68	25.79
T_{32}	$S_0F_3I_2$	15.81	24.56	17.07	23.76	16.44	24.16
T_{33}	$S_1F_3I_3$	18.33	28.18	18.38	27.04	18.35	27.61
T_{34}	$S_2F_3I_3$	16.56	25.81	16.35	26.56	16.45	26.18
T_{35}	$S_3F_3I_3$	17.96	27.68	18.18	26.06	18.07	26.87
T_{36}	$S_0F_3I_3$	13.33	19.35	13.26	16.92	13.29	18.13
	SE ±	**0.669**	**1.424**	**0.772**	**1.316**	**0.706**	**0.723**
	CD at 5%	**2.006**	**4.085**	**2.214**	**3.777**	**2.027**	**2.074**

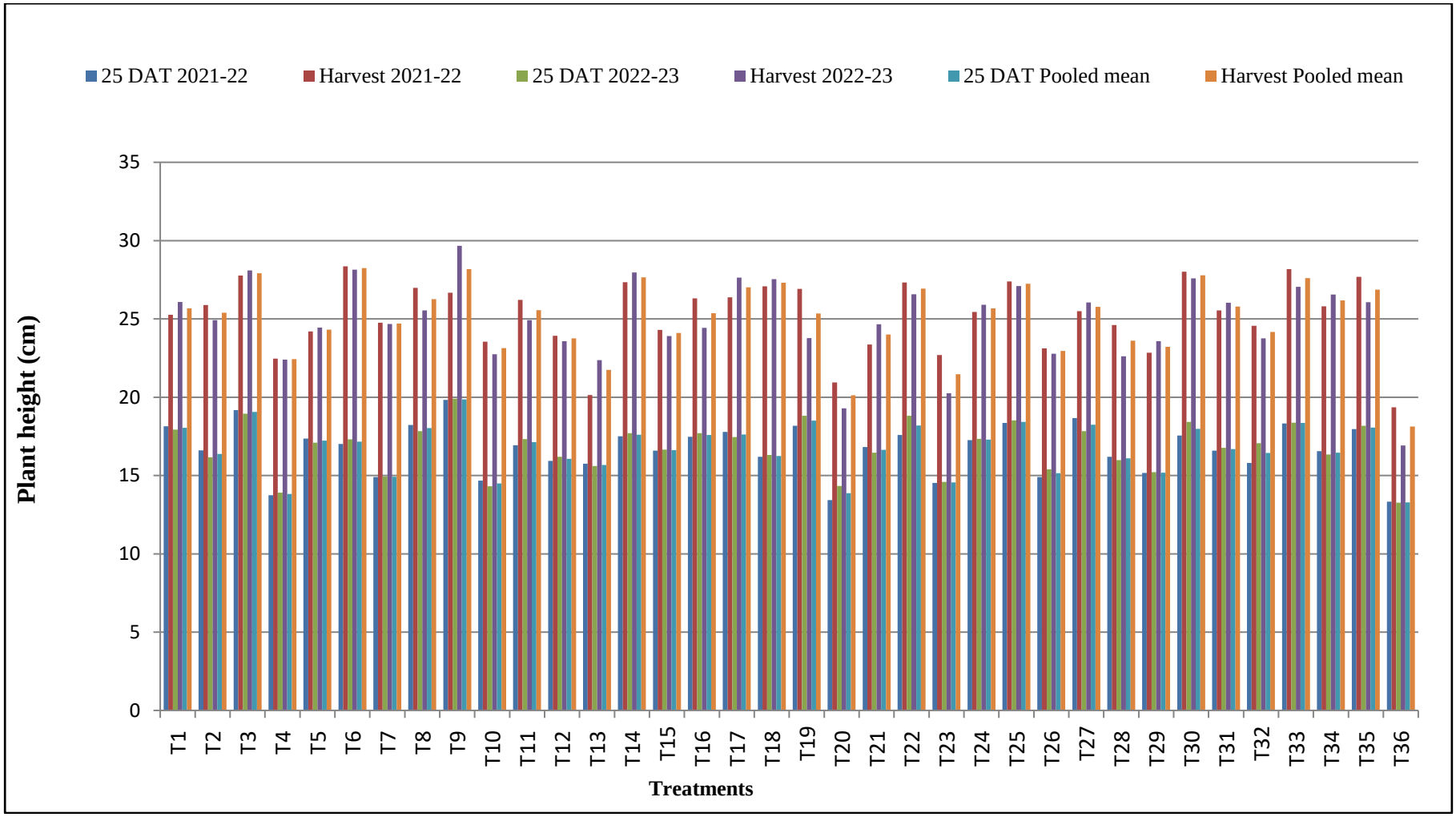

Fig. 1. Interaction effect of different substrates, levels of nutrient concentration and application time interval on plant height of lettuce

The higher plant height in the mixture of cocopeat + perlite (1:1) could be attributed to the greater physio-chemical qualities of the substrates as compared to cocopeat alone. Furthermore, addition of perlite substrate in cocopeat (1:1 v/v) have been suggested to provide several macro and micronutrients, which supports enhanced metabolism and improve structural quality of the substrate resulting in better vegetative growth in terms of plant height in lettuce. This might be due to proper aeration, better water holding capacity, lower bulk density and bio stability of treatments as compared with conventional method. Plant height varied for time and the fastest growth was determined in the perlite media (Kilic et al. 2018). These results are in agreement with the finding of Bhat et al. (2013b) in tomato, cucumber and capsicum, Olubanjo and Alade (2018) in tomato, Sadek et al. (2018) in lettuce, Rahman et al. (2019) in lettuce, Sharma (2020) in lettuce, Spehia et al. (2020) in tomato, Subramani et al. (2020) in tomato, Islam et al. (2021) in lettuce, Kalkan and Sonmez (2021) in spinach, Gaikwad et al. (2023) in capsicum and Raj et al. (2023) in lettuce.

3.2 Number of Inner Leaves Per Plant

3.2.1 Effect on number of inner leaves at 25 DAT

Among interactions, significantly the highest number of inner leaves per plant (8.50) was noticed in the treatment T_{11} [$S_3F_1I_3$] *i.e.* Cocopeat + Perlite (2:1) + 100% + Six days interval and T_{25} [$S_1F_3I_1$] *i.e.* Cocopeat + 60% + One day interval. However, lowest number of inner leaves per plant (4.85) was found with treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval in pooled mean. Further it was showed that the highest number of inner leaves per plant (9.00) was observed in the treatment T_3 [$S_3F_1I_1$] *i.e.* Cocopeat + Perlite (2:1) + 100% + One day interval and lowest (5.30) was observed with treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval during the year 2022-23. Whereas in the year 2022-23, the maximum number of inner leaves per plant (8.70) was recorded in treatment T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval, T_{17} [$S_1F_2I_2$] *i.e.* Cocopeat + 80% + Three days interval and T_{25} [$S_1F_3I_1$] *i.e.* Cocopeat + 60% + One day interval. While, minimum number of inner leaves per plant (4.40) was observed with treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval.

3.2.2 Effect on number of inner leaves at harvest

Among interactions, significantly the highest number of inner leaves per plant (18.50) was noticed in treatment T_6 [$S_2F_1I_2$] *i.e.* Cocopeat + Perlite (1:1) + 100% + Three days interval and it was found minimum (10.60) in treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval in pooled mean. Further it was noticed that the maximum number of inner leaves per plant (20.20) was recorded in the treatment combination of T_{32} [$S_0F_3I_2$] *i.e.* Soil + 60% + Three days interval and lowest (10.90) was recorded with treatment combination of T_{23} [$S_3F_2I_3$] *i.e.* Cocopeat + Perlite (2:1) + 80% + Six days interval during the year 2021-22. Whereas, the highest number of inner leaves per plant (19.00) was observed in the treatment T_9 [$S_1F_1I_3$] *i.e.* Cocopeat + 100% + Six days interval and T_{30} [$S_2F_3I_2$] *i.e.* Cocopeat + Perlite (1:1) + 60% + Three days interval. However, lowest number of inner leaves per plant (9.50) was observed in treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval during the year 2022-23.

A significant variation was found due to combined effect of substrates, levels of nutrient concentration and application time interval on number of inner leaves per plant at 25 DAT and harvest presented in Table 2 and depicted in Fig. 2 during the year 2021-22, 2022-23 and pooled mean. The treatment T_6 [$S_2F_1I_2$] recorded highest number of inner leaves of lettuce were found in plants grown in substrate mixture of cocopeat + perlite (1:1) due to supplied of 100% RDF nutrient through water soluble fertilizer (19:19:19) with application time interval of three days.

Number of inner leaves increased with sole use of cocopeat at 25 DAT or together with perlite in different volume (1:1) in combined effect at harvest than soil. This might be attributed to synergistic effect of different substrates on plant growth and development. The mixture of this two substrate improve the structural quality and provide sufficient nutrients at specific time interval result in increase the number of inner leaves. Finding corroborates with their result obtained by Thapa et al. (2016) in sweet pepper and Makhadmeh et al. (2017) in lettuce.

In three-way interaction effect, there were significant effect of levels of nutrient concentration on number of inner leaves per plant at all the growth stage of lettuce plant. Each incremental dose of fertilizers caused increase in

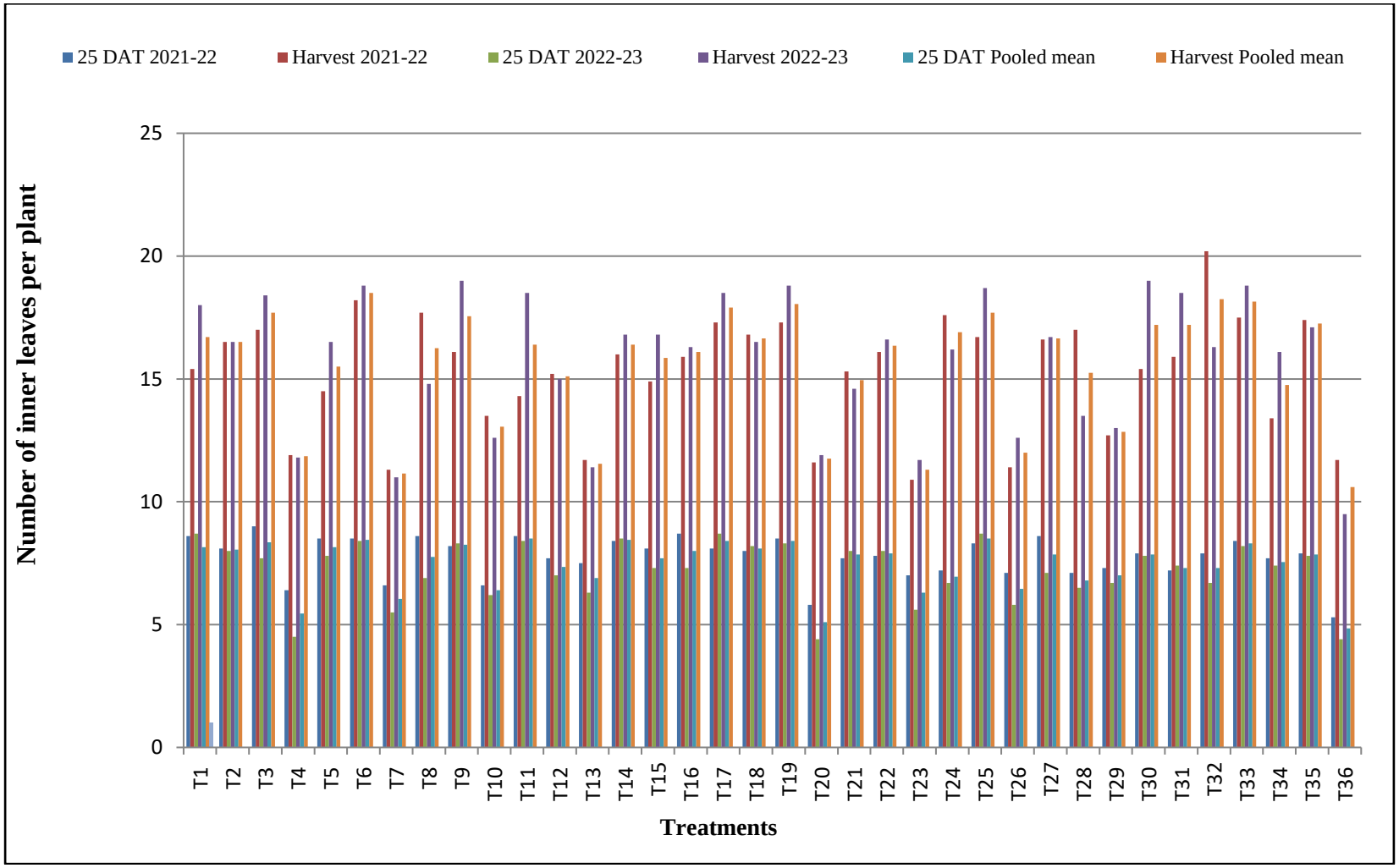

Fig. 2. Interaction effect of different substrates, levels of nutrient concentration and application time interval on number of inner leaves per plant of lettuce

Table 2. Interaction effect of different substrates, levels of nutrient concentration and application time interval on number of inner leaves per plant of lettuce

Treatment No.	Treatments	Number of inner leaves per plant					
		2021-22		2022-23		Pooled mean	
		25 DAT	Harvest	25 DAT	Harvest	25 DAT	Harvest
	Interaction: S × F × I						
T_1	$S_1F_1I_1$	8.60	15.40	8.70	18.00	8.15	16.70
T_2	$S_2F_1I_1$	8.10	16.50	8.00	16.50	8.05	16.50
T_3	$S_3F_1I_1$	9.00	17.00	7.70	18.40	8.35	17.70
T_4	$S_0F_1I_1$	6.40	11.90	4.50	11.80	5.45	11.85
T_5	$S_1F_1I_2$	8.50	14.50	7.80	16.50	8.15	15.50
T_6	$S_2F_1I_2$	8.50	18.20	8.40	18.80	8.45	18.50
T_7	$S_3F_1I_2$	6.60	11.30	5.50	11.00	6.05	11.15
T_8	$S_0F_1I_2$	8.60	17.70	6.90	14.80	7.75	16.25
T_9	$S_1F_1I_3$	8.20	16.10	8.30	19.00	8.25	17.55
T_{10}	$S_2F_1I_3$	6.60	13.50	6.20	12.60	6.40	13.05
T_{11}	$S_3F_1I_3$	8.60	14.30	8.40	18.50	8.50	16.40
T_{12}	$S_0F_1I_3$	7.70	15.20	7.00	15.00	7.35	15.10
T_{13}	$S_1F_2I_1$	7.50	11.70	6.30	11.40	6.90	11.55
T_{14}	$S_2F_2I_1$	8.40	16.00	8.50	16.80	8.45	16.40
T_{15}	$S_3F_2I_1$	8.10	14.90	7.30	16.80	7.70	15.85
T_{16}	$S_0F_2I_1$	8.70	15.90	7.30	16.30	8.00	16.10
T_{17}	$S_1F_2I_2$	8.10	17.30	8.70	18.50	8.40	17.90
T_{18}	$S_2F_2I_2$	8.00	16.80	8.20	16.50	8.10	16.65
T_{19}	$S_3F_2I_2$	8.50	17.30	8.30	18.80	8.40	18.05
T_{20}	$S_0F_2I_2$	5.80	11.60	4.40	11.90	5.10	11.75
T_{21}	$S_1F_2I_3$	7.70	15.30	8.00	14.60	7.85	14.95
T_{22}	$S_2F_2I_3$	7.80	16.10	8.00	16.60	7.90	16.35
T_{23}	$S_3F_2I_3$	7.00	10.90	5.60	11.70	6.30	11.30
T_{24}	$S_0F_2I_3$	7.20	17.60	6.70	16.20	6.95	16.90
T_{25}	$S_1F_3I_1$	8.30	16.70	8.70	18.70	8.50	17.70
T_{26}	$S_2F_3I_1$	7.10	11.40	5.80	12.60	6.45	12.00
T_{27}	$S_3F_3I_1$	8.60	16.60	7.10	16.70	7.85	16.65
T_{28}	$S_0F_3I_1$	7.10	17.00	6.50	13.50	6.80	15.25
T_{29}	$S_1F_3I_2$	7.30	12.70	6.70	13.00	7.00	12.85
T_{30}	$S_2F_3I_2$	7.90	15.40	7.80	19.00	7.85	17.20
T_{31}	$S_3F_3I_2$	7.20	15.90	7.40	18.50	7.30	17.20
T_{32}	$S_0F_3I_2$	7.90	20.20	6.70	16.30	7.30	18.25
T_{33}	$S_1F_3I_3$	8.40	17.50	8.20	18.80	8.30	18.15
T_{34}	$S_2F_3I_3$	7.70	13.40	7.40	16.10	7.55	14.75
T_{35}	$S_3F_3I_3$	7.90	17.40	7.80	17.10	7.85	17.25
T_{36}	$S_0F_3I_3$	5.30	11.70	4.40	9.50	4.85	10.60
	SE ±	**0.478**	**1.098**	**0.642**	**1.547**	**0.481**	**1.188**
	CD at 5%	**1.372**	**3.149**	**1.843**	**4.437**	**1.381**	**3.410**

number of inner leaves per plant. It concluded that application of NPK fertilizer exerted the positive effect of plant growth characters which may be due to the role of nitrogen in chlorophyll structure which is responsible for photosynthesis and manufacture of food material in the plants. It promotes the leaf and vegetative growth (Bungard et al*.* 1999). The results are in line with those reported by Alkhader et al*.* (2013) in lettuce and Al-tawaha et al*.* (2018) in lettuce.

3.3 Number of Outer Leaves Per Plant

3.3.1 Effect on number of outer leaves at 25 DAT

Interaction effect of substrates, levels of nutrient concentration and application time interval on number of outer leaves per plant of lettuce in the year 2021-22, 2022-23 and pooled mean were found to be non-significant.

3.3.2 Effect on number of outer leaves at harvest

Among interactions, significantly the maximum number of outer leaves per plant (5.35) was found in treatment T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval and T_5 [$S_1F_1I_2$] *i.e.* Cocopeat + 100% + Three days interval.

While, minimum number of outer leaves per plant (3.55) was found in treatment T_{13} [$S_1F_2I_1$] *i.e.* Cocopeat + 80% + One day interval in pooled mean. Further it was noticed that the maximum number of outer leaves per plant (5.60) was recorded in the treatment combination of T_{19} ($S_3F_2I_2$] *i.e.* Cocopeat + Perlite (2:1) + 80% + Three days interval however, minimum number of outer leaves per plant (3.50) was recorded with both the treatment combination of T_{20} [$S_0F_2I_2$] *i.e.* Soil + 80% + Three days interval and T_{23} ($S_3F_2I_3$] *i.e.* Cocopeat + Perlite (2:1) + 80% + Six days interval during the year 2022-23.

The above results showed that there were significant difference due to combined effect of different substrates, levels of nutrient concentration and application time interval on number of outer leaves per plant in the year 2022-23 and pooled mean at harvest presented in Table 3 and depicted in Fig. 3. Lettuce with highest number of outer leaves have more value due to size of leaves which give more weight and more marketable return. Maximum number of outer leaves per plant in pooled mean data at harvest noticed with treatment T_1 [$S_1F_1I_1$] and T_5 [$S_1F_1I_2$] compared to treatment T_{13} [$S_1F_2I_1$] which recorded lowest number of outer leaves.

Leaf number was affected by substrate and nutrient concentration. The maximum number of outer leaves was found in cocopeat with different levels of nutrient solution. This might be due to cocopeat has high capacity to hold applied nutrient solution, better root growth, reduces bulk density, increases available water content and aeration promote leaf growth. Similar finding have been reported by Giil et al. (2005) in lettuce, Kumari (2013) in lettuce, Makhadmeh et al. (2017) in lettuce and Kilic et al. (2018) in tomato.

Number of outer leaves increased when application levels of fertilizer dose increased with their application time interval changes. It is noticed that maximum number of outer leaves were produced due to application of fertilizer 100% RDF at one or three days interval in substrates cocopeat may be due to the balanced supply of nutrient at specific time interval and optimum water holding capacity of substrate in an optimum greenhouse environment. These results are in accordance with those reported by Alkhader et al. (2013) in lettuce, Mirdad (2016) in lettuce and Al-tawaha et al. (2018) in lettuce.

3.4 Leaf Area (cm^2)

3.4.1 Effect on leaf area at 25 DAT

Among interactions, significantly the highest leaf area (54.66) was recorded in treatment T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval and the lowest leaf area (23.71) was recorded in treatment T_{20} [$S_0F_2I_2$] *i.e.* Soil + 80% + Three days interval in pooled mean. Similarly it was noticed that the maximum leaf area (57.78) was also observed in treatment T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval and minimum leaf area (20.55) was observed with treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval during the year 2022-23. Further it was resulted that the highest leaf area (56.34) was noticed in the treatment T_9 [$S_1F_1I_3$] *i.e.* Cocopeat + 100% + Six days interval whereas, the lowest leaf area (26.63) was noticed with treatment T_{20} [$S_0F_2I_2$] *i.e.* Soil + 80% + Three days interval during the year 2021-22.

3.4.2 Effect on leaf area at harvest

Among interactions, significantly the highest leaf area (105.84) was observed in treatment T_{33} ($S_1F_3I_3$] *i.e.* Cocopeat + 60% + Six days interval which was followed by treatment T_{17}, T_1, T_{11}, T_9 and T_6. Whereas, the lowest leaf area (57.35) was observed with treatment T_{36} [$S_0F_3I_3$] *i.e.* Soil + 60% + Six days interval in pooled mean. Further it was noticed that the highest leaf area (106.65) was recorded in the treatment combination of T_{17} [$S_1F_2I_2$] *i.e.* Cocopeat + 80% + Three days interval and the lowest leaf area (58.32) was found with treatment combination of T_{10} [$S_2F_1I_3$] *i.e.* Cocopeat + Perlite (1:1) + 100% + Six days interval during the year 2021-22. Whereas, it was resulted that the maximum leaf area (106.72) was recorded in the treatment combination of T_1 [$S_1F_1I_1$] *i.e.* Cocopeat + 100% + One day interval which was followed by treatment T_{33} ($S_1F_3I_3$] (106.06), T_9 ($S_1F_1I_3$] (103.01), T_{17} ($S_1F_2I_2$] (102.78), T_{14} ($S_2F_2I_1$] (99.55), T_{11} ($S_3F_1I_3$] (98.70) and T_6 ($S_2F_1I_2$] (98.35). However, minimum leaf area (52.65) was recorded with treatment combination of T_{20} [$S_0F_2I_2$] *i.e.* Soil + 80% + Three days interval during the year 2022-23.

Table 3. Interaction effect of different substrates, levels of nutrient concentration and application time interval on number of outer leaves per plant of lettuce

Treatment No.	Treatments	Number of outer leaves per plant					
		2021-22		2022-23		Pooled mean	
		25 DAT	Harvest	25 DAT	Harvest	25 DAT	Harvest
	Interaction: S × F × I						
T_1	$S_1F_1I_1$	4.90	5.80	4.30	4.90	4.60	5.35
T_2	$S_2F_1I_1$	4.80	5.40	3.90	4.60	4.35	5.00
T_3	$S_3F_1I_1$	4.70	5.00	4.40	4.60	4.55	4.80
T_4	$S_0F_1I_1$	3.60	3.40	4.00	4.30	3.80	3.85
T_5	$S_1F_1I_2$	4.70	5.60	4.20	5.10	4.45	5.35
T_6	$S_2F_1I_2$	4.70	5.40	4.20	4.80	4.45	5.10
T_7	$S_3F_1I_2$	4.40	4.00	4.10	3.70	4.25	3.85
T_8	$S_0F_1I_2$	4.20	4.60	4.40	4.40	4.30	4.50
T_9	$S_1F_1I_3$	4.50	4.90	3.90	4.50	4.20	4.70
T_{10}	$S_2F_1I_3$	4.70	4.60	3.90	4.10	4.30	4.35
T_{11}	$S_3F_1I_3$	4.10	4.50	4.50	4.30	4.30	4.40
T_{12}	$S_0F_1I_3$	4.60	4.80	4.70	4.30	4.65	4.55
T_{13}	$S_1F_2I_1$	4.70	3.50	3.80	3.60	4.25	3.55
T_{14}	$S_2F_2I_1$	4.60	4.50	4.30	4.90	4.45	4.70
T_{15}	$S_3F_2I_1$	4.40	5.00	5.00	5.30	4.70	5.15
T_{16}	$S_0F_2I_1$	4.00	4.40	4.60	4.00	4.30	4.20
T_{17}	$S_1F_2I_2$	4.90	5.20	4.30	4.70	4.60	4.95
T_{18}	$S_2F_2I_2$	5.00	4.70	3.80	5.30	4.40	5.00
T_{19}	$S_3F_2I_2$	4.70	5.00	4.20	5.60	4.45	5.30
T_{20}	$S_0F_2I_2$	4.00	3.80	4.10	3.50	4.05	3.65
T_{21}	$S_1F_2I_3$	4.50	5.10	4.20	5.20	4.35	5.15
T_{22}	$S_2F_2I_3$	4.90	3.80	3.50	5.30	4.20	4.55
T_{23}	$S_3F_2I_3$	4.20	4.00	4.10	3.50	4.15	3.75
T_{24}	$S_0F_2I_3$	4.30	4.00	4.80	4.30	4.55	4.15
T_{25}	$S_1F_3I_1$	5.00	5.10	4.40	4.30	4.70	4.70
T_{26}	$S_2F_3I_1$	4.80	4.10	3.70	4.40	4.25	4.25
T_{27}	$S_3F_3I_1$	4.90	4.00	4.70	5.00	4.80	4.50
T_{28}	$S_0F_3I_1$	3.90	4.80	4.60	4.00	4.25	4.40
T_{29}	$S_1F_3I_2$	4.70	4.40	3.60	4.50	4.15	4.45
T_{30}	$S_2F_3I_2$	5.00	4.50	4.50	4.20	4.75	4.35
T_{31}	$S_3F_3I_2$	4.80	4.50	4.60	4.70	4.70	4.60
T_{32}	$S_0F_3I_2$	4.00	4.80	4.70	4.40	4.35	4.60
T_{33}	$S_1F_3I_3$	4.70	5.10	4.60	4.20	4.65	4.65
T_{34}	$S_2F_3I_3$	4.60	4.70	4.60	5.00	4.60	4.85
T_{35}	$S_3F_3I_3$	4.30	4.30	4.40	5.30	4.35	4.80
T_{36}	$S_0F_3I_3$	3.90	3.80	3.80	3.60	3.85	3.70
	SE ±	**0.240**	**0.539**	**0.389**	**0.424**	**0.258**	**0.398**
	CD at 5%	**NS**	**NS**	**NS**	**1.217**	**NS**	**1.142**

A significant variation was found due to combined effect of substrates, levels of nutrient concentration and application time interval at 25 DAT and harvest as given in Table 4 and shows in Fig. 4 during the year 2021-22, 2022-23 and pooled mean. The treatment T_1 [$S_1F_1I_1$] observed highest leaf area of lettuce (54.66 cm^2) at 25 DAT and treatment T_{33} [$S_1F_3I_3$] observed highest leaf area of lettuce (105.84 cm^2) at harvest in pooled mean data which was at par with T_6, T_9, T_{11} and T_{17}.

A positive direct effect of leaf area was observed on leaf yield per plant. The above results showed that the leaf area significantly varied among the different treatments at 25 DAT and at harvest. The different substrates induced significantly positive impact on leaf area, especially using of substrate S_1 recorded highest leaf area of lettuce followed by S_2 and S_3 during both the season as well as in pooled data. The highest leaf area was noticed in cocopeat with different levels of nutrient solution might be due to good

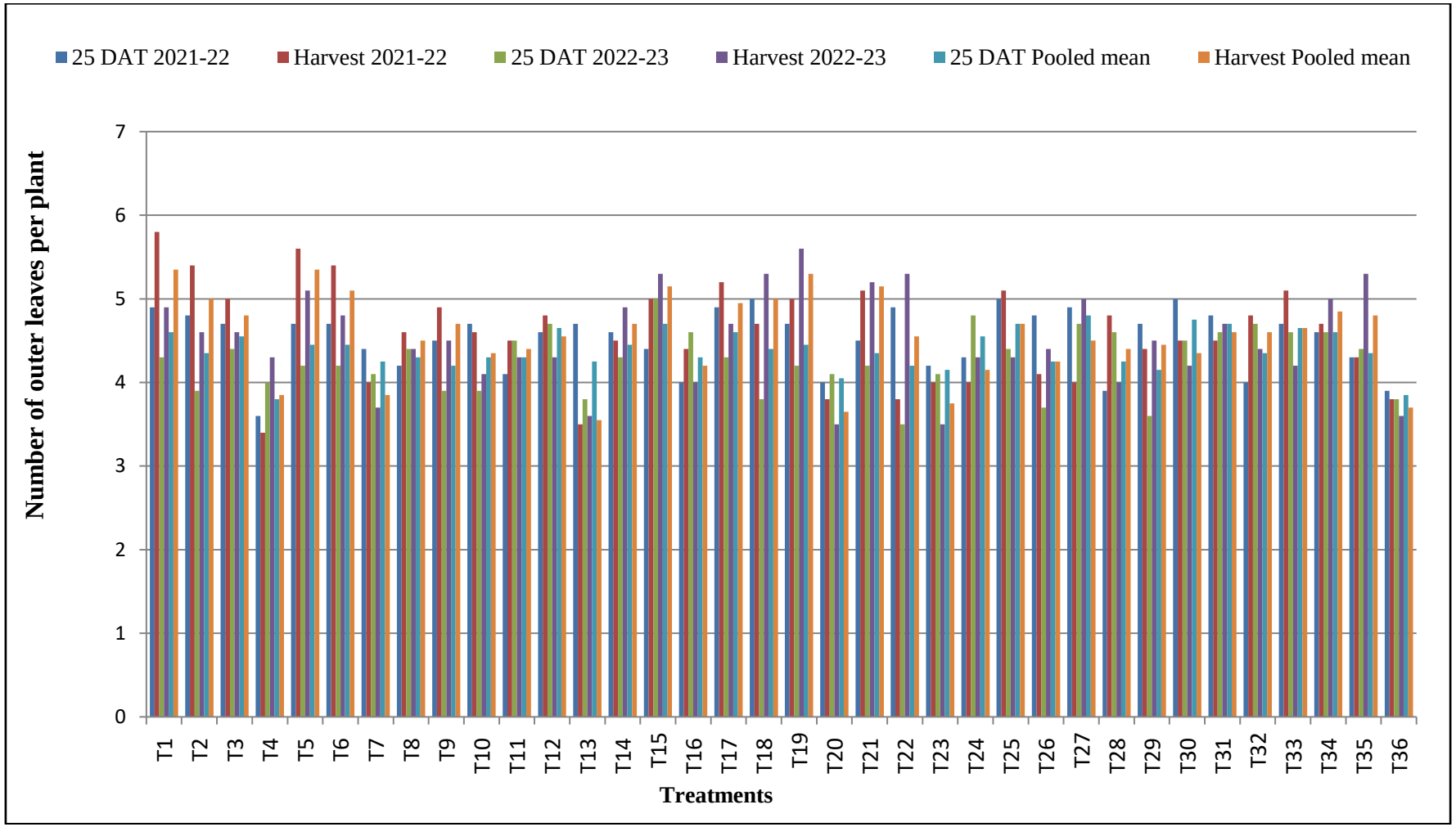

Fig. 3. Interaction effect of different substrates, levels of nutrient concentration and application time interval on number of outer leaves per plant of lettuce

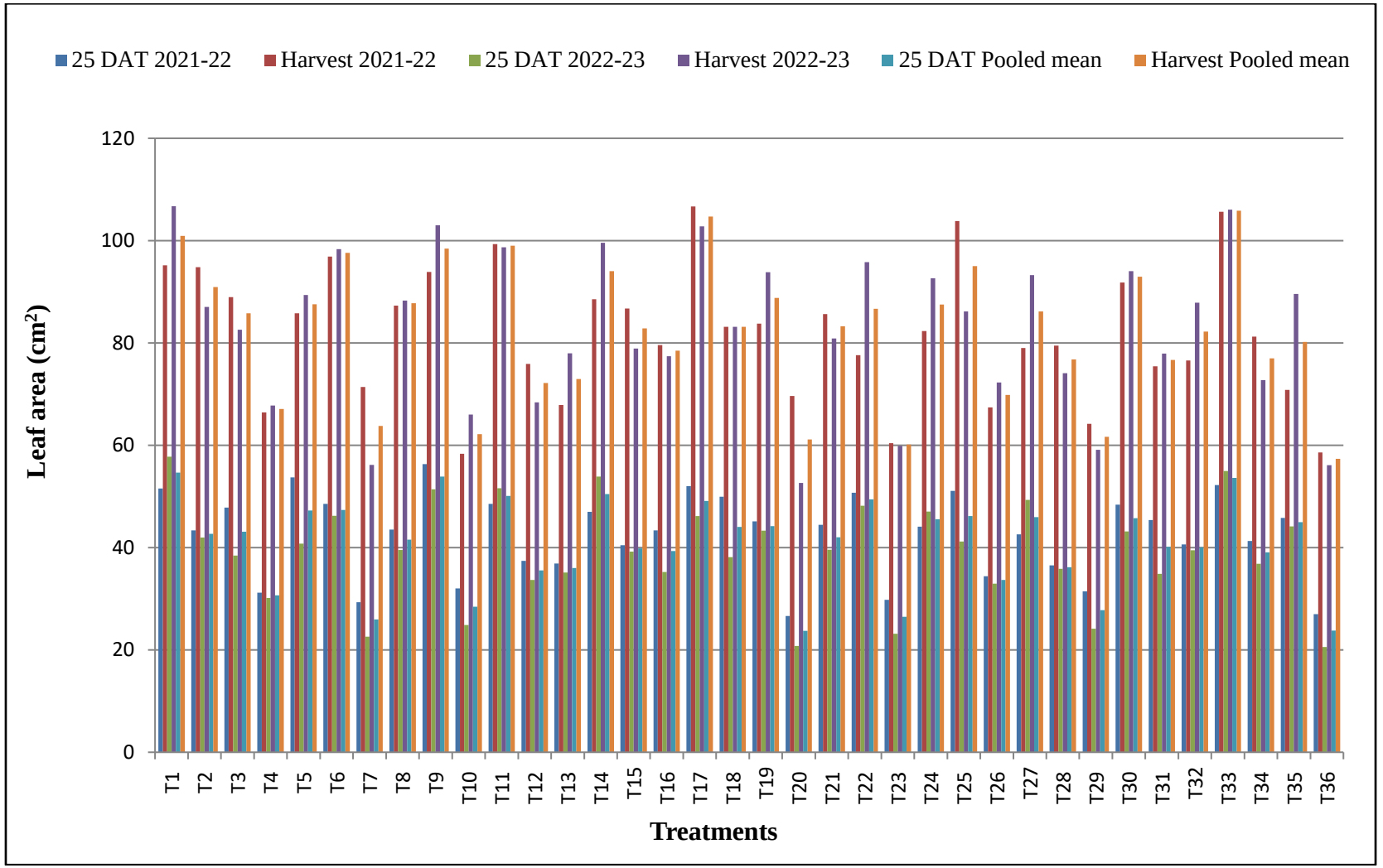

Fig. 4. Interaction effect of different substrates, levels of nutrient concentration and application time interval on leaf area of lettuce

Table 4. Interaction effect of different substrates, levels of nutrient concentration and application time interval on leaf area of lettuce

Treatment No.	Treatments	Leaf area (cm^2)					
		2021-22		2022-23		Pooled mean	
		25 DAT	Harvest	25 DAT	Harvest	25 DAT	Harvest
	Interaction: S × F × I						
T_1	$S_1F_1I_1$	51.53	95.17	57.78	106.72	54.66	100.94
T_2	$S_2F_1I_1$	43.38	94.79	41.95	87.04	42.67	90.91
T_3	$S_3F_1I_1$	47.82	88.95	38.44	82.60	43.13	85.78
T_4	$S_0F_1I_1$	31.20	66.44	30.14	67.76	30.67	67.10
T_5	$S_1F_1I_2$	53.73	85.79	40.76	89.37	47.25	87.58
T_6	$S_2F_1I_2$	48.53	96.90	46.22	98.35	47.37	97.63
T_7	$S_3F_1I_2$	29.30	71.40	22.61	56.17	25.95	63.79
T_8	$S_0F_1I_2$	43.52	87.28	39.55	88.29	41.53	87.78
T_9	$S_1F_1I_3$	56.34	93.85	51.41	103.01	53.87	98.42
T_{10}	$S_2F_1I_3$	32.03	58.32	24.86	66.01	28.45	62.16
T_{11}	$S_3F_1I_3$	48.54	99.33	51.62	98.70	50.08	99.02
T_{12}	$S_0F_1I_3$	37.43	75.91	33.66	68.39	35.54	72.15
T_{13}	$S_1F_2I_1$	36.89	67.88	35.13	77.99	36.01	72.93
T_{14}	$S_2F_2I_1$	47.01	88.52	53.89	99.55	50.45	94.04
T_{15}	$S_3F_2I_1$	40.44	86.75	39.21	78.92	39.83	82.83
T_{16}	$S_0F_2I_1$	43.37	79.57	35.24	77.42	39.30	78.49
T_{17}	$S_1F_2I_2$	52.04	106.65	46.17	102.78	49.10	104.72
T_{18}	$S_2F_2I_2$	49.95	83.16	38.11	83.14	44.03	83.15
T_{19}	$S_3F_2I_2$	45.12	83.79	43.29	93.81	44.20	88.80
T_{20}	$S_0F_2I_2$	26.63	69.64	20.80	52.65	23.71	61.15
T_{21}	$S_1F_2I_3$	44.44	85.65	39.58	80.88	42.01	83.27
T_{22}	$S_2F_2I_3$	50.71	77.63	48.16	95.77	49.44	86.70
T_{23}	$S_3F_2I_3$	29.78	60.39	23.14	59.91	26.46	60.15
T_{24}	$S_0F_2I_3$	44.10	82.33	47.03	92.63	45.56	87.48
T_{25}	$S_1F_3I_1$	51.11	103.84	41.21	86.15	46.16	94.99
T_{26}	$S_2F_3I_1$	34.41	67.40	32.96	72.27	33.68	69.83
T_{27}	$S_3F_3I_1$	42.57	79.02	49.31	93.25	45.94	86.14
T_{28}	$S_0F_3I_1$	36.51	79.46	35.85	74.09	36.18	76.78
T_{29}	$S_1F_3I_2$	31.43	64.18	24.13	59.11	27.78	61.65
T_{30}	$S_2F_3I_2$	48.37	91.81	43.14	94.05	45.75	92.93
T_{31}	$S_3F_3I_2$	45.39	75.42	34.89	77.93	40.14	76.67
T_{32}	$S_0F_3I_2$	40.60	76.58	39.46	87.89	40.04	82.23
T_{33}	$S_1F_3I_3$	52.22	105.61	54.98	106.06	53.60	105.84
T_{34}	$S_2F_3I_3$	41.30	81.23	36.82	72.74	39.06	76.98
T_{35}	$S_3F_3I_3$	45.81	70.82	44.14	89.58	44.97	80.20
T_{36}	$S_0F_3I_3$	27.01	58.58	20.55	56.11	23.78	57.35
	SE ±	**4.339**	**5.433**	**2.802**	**3.004**	**3.102**	**3.712**
	CD at 5%	**12.448**	**15.587**	**8.038**	**8.618**	**8.899**	**10.649**

physio-chemical properties of substrate, high total pore space, high moisture content, low shrinkage, reduces bulk density and capacity to hold applied nutrient solution result that the roots will absorb nutrient needed by the plants in vegetative growth so that the lettuce plant produces shoot that will develop into leaves and leaf growth will changes rapidly which caused more leaf area. This is mainly due to increased macro and micronutrient absorption which stimulates the metabolic processes and the accumulation of additional metabolites in plant tissue. The present results are also in-line with the findings of Arenas et al*.* (2002) in tomato, Awang et al*.* (2009) in celosia, Islam et al*.* (2021) in lettuce and Gaikwad et al*.* (2023) in capsicum.

In three-way interaction effect, there were significant effect of levels of nutrient concentration and their application time interval on leaf area at all the growth stage of lettuce plant. The increase in growth parameter as response to increased fertilization is probably due to enhanced availability of N, P and K in the substrate medium which enhanced leaf area resulting in higher photoassimilates. The increased leaf area might be due to increased translocation of nutrient to the aerial parts for synthesis of protoplasmic protein and other metabolic activities which lead top increase in various plant metabolites responsible for cell division and elongation and increasing photosynthetic area in an optimum environment condition. The present results are in harmony with Abgad et al*.* (2015) in spinach, Koppad et al*.* (2019) in cabbage, Kharade et al*.* (2021) in lettuce, Patel et al*.* (2021) in spinach and Nath et al*.* (2023) in lettuce.

4. CONCLUSION

It can be concluded that application of treatment T_6 [$S_2F_1I_2$] *i.e.* Cocopeat + Perlite (1:1) + 100% + Three days interval performed best over all the other treatments for most of the plant growth parameter in leafy lettuce followed by treatments T_9, T_2, T_1, T_{11}, T_5, T_{17}, T_{21}, T_{25} and T_{33}.

This should call for further investigation. As these results are based on two research trials, it is suggested to conduct a few more trials to arrive at a concrete conclusion.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author (Jadhav S. D.) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have NOT been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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