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Navyashree R
University of Agriculture Science, Dharwad, Karnataka, India

Ashvathama VH
College of Agriculture, Vijaypura, UAS, Dharwad, Karnataka, India

MD Patil
University of Agriculture Science, Dharwad, Karnataka, India

Mummigatti UV
University of Agriculture Science, Dharwad, Karnataka, India

Kiran BO
University of Agriculture Science, Dharwad, Karnataka, India

Corresponding Author:
Navyashree R
University of Agriculture Science, Dharwad, Karnataka, India

Effect of salinity stress on Morphophysiological traits in chickpea genotypes

Navyashree R, Ashvathama VH, MD Patil, Mummigatti UV and Kiran BO

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Abstract
Chickpea is grown under a wide range of climatic conditions and is known to be highly sensitive to salinity stress. An experiment was conducted during 2019-20 to study the morpho-physiological responses of chickpea (*Cicer arietinum* L.) genotypes to salinity stress, in the Department of Crop Physiology at College of Agriculture, Vijayapur. The present study was carried out with ten chickpea genotypes sown under three salinity concentration (0 dSm^{-1}, 3 dSm^{-1} and 6 dSm^{-1}). All the parameters were recorded at 30, 60 and 90 Days after sowing (DAS). The results indicated that, plant height, relative water content (RWC), SPAD value and membrane injury index were decreased with increase in salinity concentration from 3 dS/m to 6 dS/m. Maximum plant height was observed in 0 dSm^{-1} salt concentration for all genotypes. Among the genotypes significantly higher Relative Water Content (RWC) was recorded in JG11. While, lowest RWC was recorded in genotype ICCV96029 at 60 days after sowing. From the study it was observed that the genotypes JG 11, BGD 103, MNK 1 and ICC 1431 were tolerant to salinity while, ICCV 96029 and NBeG 47 were found to be salt sensitive genotypes.

Keywords: Chickpea, salinity, RWC and plant height

Introduction
The chickpea cultivation is mainly concentrated in semi-arid environments such as South Asia, West Asia, North Africa, East Africa, Southern Europe, North and South America and Australia (Arefian, *et al.*, 2014) [2]. The cultivated chickpea species are grouped into *desi* and *Kabuli* types. *Desi types* have small and darker brown coloured seeds, whereas *kabuli* types are bold seeded and cream-coloured.
Soil salinity is known as a major inevitable problem, especially in arid and semi-arid regions of the world, where these regions are the main cultivation areas of chickpea (Flowers, *et al.*, 2010) [6]. Legumes, especially cool-season food legumes like chickpea, lentil and faba bean are relatively sensitive to soil salinity (Sheldon *et al.*, 2004) [14]. From agriculture point of view soils with high salt concentration that adversely affect plant growth and crop productivity are called salt affected soils. Saline soil is formed when chlorides and sulphates of sodium, calcium, magnesium and potassium are abundant in the soil and the process is known as salinization.
Salinity stress adversely affects several morphological features and physiological processes like reduction in growth, decrease in chlorophyll, ion imbalance, water stress, decreased photosynthesis, increase in hydrogen peroxide, which causes lipid per oxidation and consequently membrane injury, reduced nodulation and N_2 fixation (Zhu, 2001) [16]. Keeping these in view this study is designed to assess the germination, physiological and morphological responses in chickpea under salt stress.

Materials and Methods
Experimental site
The College of Agriculture, Vijayapura is situated at 16°49’ N latitude and 76°34’ E longitude with an altitude of 678 meters above the mean sea level (MSL). The pot experiment was conducted at rain out shelter, College of Agriculture, Vijayapur and laboratory study was conducted at Department of Crop Physiology.

Salinity treatments

Screening of 10 varieties of chickpea was carried out for sodium chloride salinity. The solutions represented a grade series consisting of C1=0, C2=3 and C3=6 dSm^{-1} of NaCI salt. The above salt solutions were prepared by following methods given in the U.S.D.A Hand book No.60 (Richards, 1954).

Details of the experiment

The experiment was conducted during the year 2019-20 (pot experiment) to evaluation of 10 chickpea genotypes with two salinity levels and control treatment involving screening of genotypes for salinity tolerance. The data on morphological, and physiological associated traits were recorded at timely intervals in pot experiment.

Sowing and salinity treatments

Plastic pots of uniform size (30 x 30 cm) were filled with 10 kg of air-dried soil and farmyard manure in 6:1 ratio. Before sowing pots were irrigated with 2.5 litters of water (control) or salt solutions of different concentrations. The plants were subjected to three conditions *viz.* control (C1) and two salinity treatments (C2 and C3). Salt solutions were prepared by using NaCl salt. The salt concentrations of different solutions are given below.

C2 = 5 gram of NaCl salt dissolved in 1 litter of water for preparing 3 EC

C3 = 10 gram of NaCl salt dissolved in 1 litter of water for preparing 6 EC

Actual salinity values are expressed as EC determined at three stages and mean of these was taken as salinity at C1, C2 and C3 levels.

Morphological and physiological parameters

Plant Height

Plant height of randomly selected three plants was recorded in centimeter by measuring length from the base of the plant near the soil surface up to the growing point of the longest branch in three plants at 30, 60 and 90 days after sowing.

Relative Water Content (%)

Leaf relative water content was estimated by recording the turgid weight of fresh leaf samples by keeping in water for four hours, followed by drying in hot air oven till constant weight is achieved. RWC (%) was calculated by the formula given by Kramer (1983) [10].

$$\text{RWC (\%)} = \frac{\text{Fresh Weight (g)} - \text{Dry Weight (g)}}{\text{Turgid Weight (g)} - \text{Dry Weight (g)}} \times 100$$

SPAD values

The chlorophyll meter or SPAD meter is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves (Inada, 1963 and Kariya *et al.*, 1982) [8, 9]. The observations recorded in three plants per treatment at 30 days, 60 days and 90 days after sowing and expressed in terms of SPAD values.

Membrane injury index

Membrane injury index was measured as percent proportion of ion leakage in to the external aqueous medium to the total ion concentration of the stressed tissue as measured by the EC of the external medium (Sullivan and Ross, 1979) [15].

Procedure

The 200 mg of leaf were kept in 20 ml vials containing 10 ml de-ionized water at 27 °C. After 5 h, the electrical conductivity (EC) of the surrounding solution was measured and designated as EC1. Then the samples were kept in boiling water bath for 50 min to achieve total killing of the tissue. After cooling, the EC of the solution was again measured and designated as EC2. The membrane injury index was calculated as follows:

Membrane injury index (%) = EC1 / EC2 x 100

Results

Plant height (cm)

Plant height differed significantly at 30, 60 and 90 days after sowing with respect to salinity levels and chickpea genotypes (Table 1). The genotype MNK1 (*Kabuli* type) recorded significantly maximum plant height compared to *desi* types (39.62 cm). Among *desi* genotypes BGD103 recorded maximum plant height followed by JG11 which were found on par with each other (38.64, and 38.26 cm, respectively) and the genotype ICCV96029 (32.17cm) was recorded significantly lower plant height at 90 days after sowing.

SPAD values

There was significant difference in SPAD values with respect to the genotypes, salinity levels and their interactions at 30, 60 and 90 days after sowing (table 2). Among the salinity levels, 0 dSm^{-1} recorded significantly higher SPAD values (30.20) at 30 days after sowing followed by 3 dSm^{-1} and 6dSm^{-1} (27.47 and 23.97, respectively) and similar trend was followed at 60 and 90 days after sowing. Among the genotypes, BGD103 recorded significantly higher SPAD value (46.11), followed by MNK1 and JG11 (45.67 and 45.44, respectively) at 60 days after sowing and the genotype ICCV96029 (41.56) recorded lowest SPAD value during 60 days.

Relative water content

The data on relative water content (RWC) at 30, 60 and 90 days after sowing in chickpea genotypes is represented in Table 3. Among the genotypes, JG11 was recorded significantly higher RWC (82.41%), followed by BGD103 (80.84%) which was on par with genotype MNK1 and lower RWC was recorded in genotype ICCV96029 at 60 days after sowing. However JG11 and BGD103 were recorded significantly higher relative water content at 30, 60 and 90 days after sowing compared to other genotypes.

Membrane injury index

The data on the membrane injury index at 30, 60 and 90 days is given in table 6. The membrane injury index differed significantly with respect to different salinity levels, genotypes and their interactions. Among the different salinity levels, the membrane injury index was significantly higher in 6 dSm^{-1} (13.59%) followed by 3 dSm^{-1} and 0 dSm^{-1} (11.68% and 9.53% respectively) at 30 days. Among the genotypes, significantly higher membrane injury index was recorded in genotype ICCV96029 (15.14%) followed by JAKI9218 (14.37%) which was on par with NBeG47 at 90 days.

Table 1: Effect of salinity stress on plant height (cm) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Plant height at 30 days				Plant height at 60 days				Plant height at 90 days			
	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean
Annigeri 1	20.93	19.43	15.43	18.60	45.47	37.60	24.37	35.81	46.03	38.10	24.53	36.22
JAKI 9218	22.85	18.43	13.77	18.35	41.57	34.23	24.90	33.57	42.77	35.10	24.83	34.23
BGD 103	27.28	21.10	16.17	21.52	48.43	39.30	26.53	38.09	48.73	40.10	27.10	38.64
MNK 1	34.53	26.87	15.27	25.56	50.06	40.57	27.17	39.26	50.30	41.07	27.50	39.62
JG11	29.83	21.23	16.67	22.58	47.20	38.47	27.33	37.67	47.23	39.83	27.70	38.26
GBM 2	28.40	18.00	13.87	20.09	40.80	32.00	23.23	32.01	43.80	33.20	23.33	33.44
NBeG 47	25.30	16.90	12.20	18.13	43.60	31.23	21.70	32.18	44.13	32.80	22.23	33.06
ICC 1431	27.33	17.43	16.37	20.38	46.07	34.33	23.37	34.59	46.70	35.50	24.03	35.41
ICC 5003	25.90	18.10	14.53	19.51	42.13	33.83	25.37	33.78	43.17	35.00	25.83	34.67
ICCV 96029	25.93	14.57	10.37	16.96	48.10	28.43	18.30	31.61	48.20	29.27	19.03	32.17
Mean	26.83	19.21	14.46		45.34	35.00	24.23		46.11	36.00	24.61	
	S.E.M±		LSD @5%		S.E.M±		LSD @5%		S.E.M±		LSD @5%	
EC	0.08		0.21		0.08		0.22		0.07		0.19	
Genotypes	0.26		0.68		0.28		0.74		0.24		0.65	
Interaction (E*G)	0.77		2.05		0.83		2.21		0.73		1.94	

Table 2: Effect of salinity stress on relative chlorophyll content (SPAD) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	SPAD values at 30 days				SPAD values at 60 days				SPAD values at 90 days			
	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean
Annigeri 1	30.33	27.00	21.33	26.22	49.33	43.67	40.67	44.56	25.67	21.00	20.33	22.33
JAKI 9218	29.33	26.67	24.00	26.67	48.00	43.00	42.67	44.56	23.33	20.33	19.67	21.11
BGD 103	32.33	29.67	23.33	28.44	53.00	46.33	39.00	46.11	27.33	24.00	22.00	24.44
MNK 1	31.00	29.00	25.67	28.56	52.00	45.33	39.67	45.67	26.67	24.67	22.33	24.56
JG11	32.67	30.00	25.33	29.33	54.33	47.67	34.33	45.44	28.33	25.67	23.00	25.67
GBM 2	28.33	26.33	26.33	27.00	47.33	42.33	41.33	43.67	20.33	20.00	18.33	19.56
NBeG 47	28.00	25.67	22.33	25.33	46.67	41.67	37.67	42.00	22.67	19.67	17.67	20.00
ICC 1431	29.00	27.67	21.67	26.11	51.33	44.67	36.33	44.11	24.00	23.00	21.33	22.78
ICC 5003	33.33	28.00	24.67	28.67	50.33	44.00	35.67	43.33	26.00	22.33	21.00	23.11
ICCV 96029	27.67	24.67	25.00	25.78	45.67	40.67	38.33	41.56	19.33	19.00	16.00	18.11
Mean	30.20	27.47	23.97		49.80	43.93	38.57		24.37	21.97	20.17	
	S.E.M±		LSD @5%		S.E.M±		LSD @5%		S.E.M±		LSD @5%	
EC	0.06		0.16		0.03		0.09		0.03		0.09	
Genotypes	0.20		0.53		0.11		0.31		0.12		0.31	
Interaction (E*G)	0.60		1.60		0.34		0.92		0.35		0.93	

Table 3: Effect of salinity stress on leaf relative water content (%) at 30, 60 and 90 DASin chickpea genotypes

Genotypes	Relative water content at 30 days				Relative water content at 60 days				Relative water content at 90 days			
	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean
Annigeri 1	81.52	74.69	62.83	73.01	87.33	75.50	65.53	76.12	79.12	68.20	61.50	69.61
JAKI 9218	80.13	73.72	62.17	72.00	87.00	74.50	63.17	74.89	76.67	66.53	61.07	68.09
BGD 103	86.00	80.82	67.48	78.10	91.83	80.00	70.70	80.84	82.40	72.87	65.83	73.70
MNK 1	85.11	80.11	64.93	76.72	89.50	79.67	68.67	79.28	81.60	71.41	65.10	72.70
JG11	88.05	83.31	71.04	80.80	93.00	81.20	73.03	82.41	84.17	74.50	66.73	75.13
GBM 2	79.09	72.80	60.17	70.69	86.83	73.50	62.00	74.11	75.84	65.80	60.07	67.24
NBeG 47	78.58	72.22	59.17	69.99	84.17	72.00	60.83	72.33	77.50	65.20	59.00	67.23
ICC 1431	83.20	77.76	63.83	74.93	88.33	76.83	66.20	77.12	78.67	69.27	62.50	70.14
ICC 5003	82.40	76.40	63.17	73.99	89.33	77.67	67.67	78.22	79.32	69.67	63.57	70.85
ICCV 96029	77.79	67.90	55.33	67.01	85.67	70.33	58.70	71.57	78.00	63.64	56.17	65.94
Mean	82.19	75.97	63.01		88.30	76.12	65.65		79.33	68.71	62.15	
	S.E.M±		LSD @5%		S.E.M±		LSD @5%		S.E.M±		LSD @5%	
EC	0.09		0.23		0.12		0.32		0.13		0.34	
Genotypes	0.29		0.77		0.40		1.07		0.43		1.14	
Interaction (E*G)	0.87		2.32		1.20		3.20		1.28		3.41	

Table 4: Effect of salinity stress on membrane injury index (%) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Membrane injury index at 30 days				Membrane injury index at 60 days				Membrane injury index at 90 days			
	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean	0 dSm-1 (Control)	3 dSm-1	6 dSm-1	Mean
Annigeri 1	9.41	12.48	14.34	12.08	10.44	13.75	15.80	13.33	10.76	14.36	16.74	13.95
JAKI 9218	9.72	12.79	14.99	12.50	10.75	14.12	16.45	13.78	11.07	14.67	17.38	14.37
BGD 103	8.40	11.86	13.65	11.30	9.43	13.19	15.10	12.57	9.74	13.74	16.04	13.17
MNK 1	8.22	10.41	12.39	10.34	9.38	11.74	13.85	11.66	9.56	12.29	14.79	12.21
JG11	8.51	11.72	13.52	11.25	9.54	13.09	14.94	12.52	9.85	13.60	15.92	13.12
GBM 2	10.25	11.54	13.46	11.75	11.28	12.90	14.92	13.03	11.60	13.42	15.86	13.63
NBeG 47	10.99	11.84	13.77	12.20	12.03	13.17	15.22	13.47	12.34	13.72	16.16	14.07
ICC 1431	9.59	10.59	12.44	10.87	10.62	11.92	13.89	12.14	10.93	12.47	14.83	12.74
ICC 5003	9.43	10.02	11.88	10.44	10.47	11.35	13.33	11.72	10.78	11.90	14.28	12.32
ICCV 96029	10.78	13.52	15.49	13.26	11.72	14.85	16.95	14.51	12.12	15.40	17.89	15.14
Mean	9.53	11.68	13.59		10.57	13.01	15.04		10.88	13.56	15.99	
	S.E.M±		LSD @5%		S.E.M±		LSD @5%		S.E.M±		LSD @5%	
EC	0.005		0.013		0.006		0.016		0.005		0.014	
Genotypes	0.017		0.047		0.020		0.052		0.018		0.048	
Interaction (E*G)	0.054		0.143		0.059		0.157		0.054		0.144	

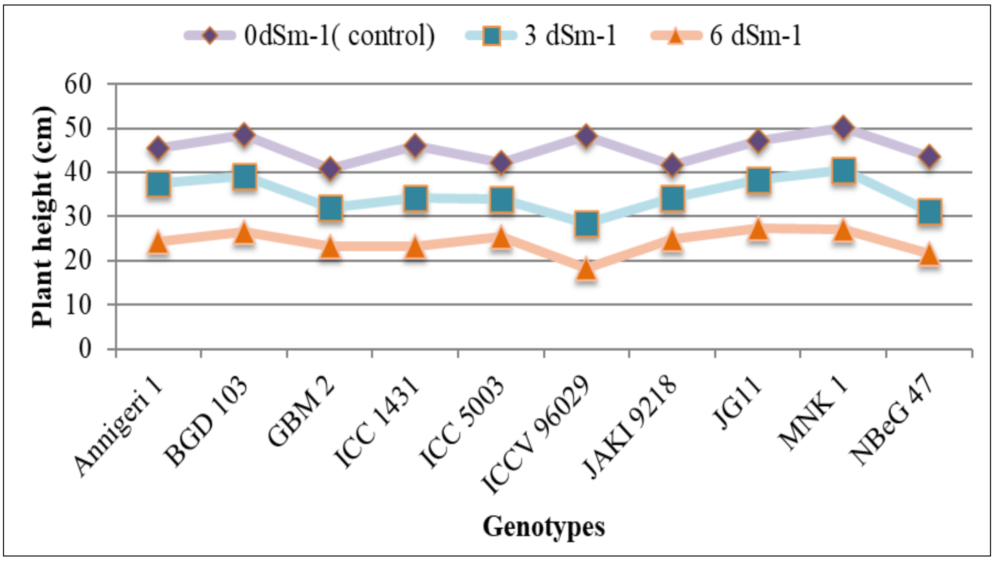

Fig 1: Effect of salinity stress on plant height of chickpea genotypes at 60 days after sowing

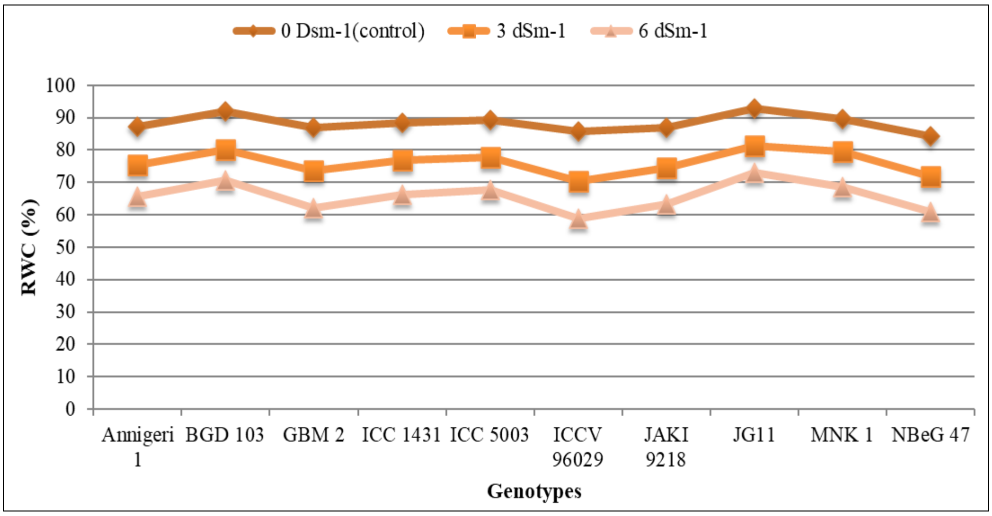

Fig 2: Effect of salinity stress on relative water content (RWC) of chickpea genotypes at 60 days after sowing

Discussion

The deleterious effects of soil salinity on plant growth and development are associated with, nutritional imbalance, low osmotic potential of soil solution (water stress) and specific ion effect (salt stress). All these factors cause adverse effect on growth and development of crops through biochemical, physiological, morphological changes like reduction in growth, ion imbalance, chlorophyll degradation, decrease in photosynthesis, reduced water status, increase in hydrogen peroxide, which causes increase in lipid peroxidation and membrane injury (Flowers *et al.*, 2010) [6]. Plants have developed various mechanisms of salinity tolerance at both inter varietal and inter specific level and even between the stages of crop growth and development including accumulation of compatible osmolytes, antioxidants and reactive oxygen species (ROS) scavenging enzymes (Ashraf and Harris, 2004) [3].

Maximum plant height, number of branches were observed in 0 dSm^{-1} salt concentration for all genotypes. The mean plant height across salinity levels was found to be 39.62 cm in MNK 1 (*Kabuli* genotype) whereas, among *desi* genotypes BGD 103 showed highest mean plant height (38.64 cm). Increased salinity level reduction of stem length and plant height due to increased salinity levels with negative effect on rate of photosynthesis, decrease in the level of carbohydrates and growth hormones and the changes in the activity of enzymes was reported by Himaya and Prapagar (2019) [7]. Rahman *et al.*, also observed on seedling height and growth of chickpea due to slow down or very less mobilization of reserve food material, suspending the cell enlargement, cell division and injuring during salinity (2008). The SPAD chlorophyll meter reading (SCMR) is an indicator of light-transmittance characteristics of the photosynthetically active leaf, which is dependent on chlorophyll density (unit amount of chlorophyll) per unit leaf area (Richardson *et al.* 2002) [13]. The water potential of leaves, RWC and osmotic potential of leaves and roots were decreased significantly under different salinity levels in chickpea genotypes (Dharamvir *et al.*, 2018). In the present investigation significantly higher RWC was recorded in 0 dSm^{-1} (88.30%) followed by 3 dSm^{-1} (76.12%) and 6 dSm^{-1} (65.65%) at 60 days after sowing.

Membrane injury index estimation gives an idea about the sensitivity of cell membrane under given set of stress conditions as it is related to leaf tissues membrane stability. Among the genotypes, significantly higher membrane injury index was recorded in ICCV96029 (15.14%). Borzouei *et al.*, (2012) [4] reported that, increased cell membrane injury and decreased membrane stability index under salt stress showed the high extent of lipid peroxidation caused by relative oxygen species. Based on the results, genotypes categorized into three groups *viz*, salt tolerant (JG11, BGD103, MNK1 and ICC1431), moderately tolerant (ICC5003, JAKI9218 and Annigeri 1) and sensitive (ICCV96029, NBeG47 and GBM2).

Conclusion
Chickpea is most important pulse crop in India and is sensitive to salt stress. Salinity stress adversely affects several morphological features and physiological processes. Plant height differed significantly not only with salinity levels and also across genotypes. Due to salt stress (3 dSm^{-1} and 6 dSm^{-1}) height of plants got reduced significantly as compare to control (0 dSm^{-1}). Physiological parameters like SPAD values, membrane injury index and relative water content (RWC) in leaves differed significantly across genotypes and salinity levels. From the study it was observed that the genotypes JG 11, BGD 103, MNK 1 and ICC 1431 were tolerant to salinity while, ICCV 96029 and NBeG 47 were found to be salt sensitive genotypes.

Author contribution
Navyashree. R., Ashvathama. V. H., Kiran. B.O. and M.D. Patil contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflict of interest
The authors declares that there is no conflict of interest.

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