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Dhanni Devi
Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

SB Gupta
Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

BK Mishra
ICAR-National Research Centre on Seed Spices, Ajmer, Rajasthan, India

Corresponding Author
Dhanni Devi
Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Effect of plant growth promoting rhizobacteria on seed germination behaviour and seedling vigour of cumin

Dhanni Devi, SB Gupta and BK Mishra

Abstract
The experiment was done at Microbiology laboratory, Division of crop production, ICAR-National Research Centre on Seed Spices, Ajmer, Rajasthan. The highest, germination percentage (86.66), seedling length (7.83 cm plant^{-1}), seedling fresh weight (145.18 mg plant^{-1}), seedling dry weight (14.03 mg plant^{-1}), seedling vigour index-I (687.5) and seedling vigour index-II (1211.16) were recorded due to seed inoculation with isolate DCU-451((*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) as compared to control.

Keywords: plant growth promoting rhizobacteria, cumin, seed germination and seed vigour

Introduction
Cumin (*Cuminum cyminum*) is a member of apiaceae family and an annual plant, which is widely cultivated in arid and semi-arid regions. It is cultivated in India during winter (rabi crop) and is a most commonly used spice/condiment in food and savouries, aroma extracts and medicinal commodity (Agrawal 2000, Pande and Goswami 2000) [1, 5]. Cumin is an important seed spices crop of western Rajasthan, which cultivating in the districts of Jaisalmer, Jalore, Pali, Barmer, Ajmer, Nagaur, Tonk and Jodhpur. Total area, total production and yield of cumin in India is 966170 ha, 688660 tonnes and 713 kg ha^{-1}, respectively, (Directorate of arecanut and spices development, Calicut, 2017-18). Individually chillies, cumin, coriander, garlic and fenugreek are the largest grown spices in the India with 23.05, 22.79, 17.48, 8.39 and 6.42 percent area and 21.88, 7.25,8.06, 23.07 and 3.56 percent production share to total spices production, respectively (Meena *et al.*, 2018) [3]. The demand for cumin is increasing in domestic as well as worldwide market which performs an critical function in national financial system. However, the production and productivity of cumin is decreasing year after year due to several reasons such as non-availability of good quality seed, low adoption of seed production technologies, poor germination, decrease the seed quality due to microbial load, heavy infestation of diseases and pests, conventional harvesting and processing, unscientific and unhygienic managing at harvest and garage are the principal hassle. Poor bodily purity and seed germination directly influences the establishment of plant populace and inflicting diseases in the field conditions. Hence, seed treatment is one of the the best method to improve seed quality and quantity also. Apart from this seed treatment with bio-inoculant not only reduces the deleterious outcomes of damage to seed viability and vigor but additionally affords better avenues for his or her status quo, growth and improvement of seedlings. Various seed enhancement treatments before sowing have additionally been devised to enhance the charge and uniform seed germination in addition to vigour in a number of crop species.

Materials and Methods
An experiment was planned for the influence of isolated potential rhizobacterial on seed germination and seed vigour index of cumin at Microbiology laboratory, Division of crop production, ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer, Rajasthan during the year 2017-18 and 2018-19. Rhizobacteria isolated from cumin plant and rhizospheric soil and samples were collected from cumin grown fields of Ajmer, Barmer, Jalore, Nagaur, Jaisalmer and Jodhpur Districts of Rajasthan. (India). On the basis of plant growth promoting traits 11 rhizobacterial isolates (DCU-22, DCU-181, DCU-184, DCU-188, DCU-251, DCU-262, DCU-351, DCU-364, DCU-451, DCU-453 and DCU-651) were screened and characterized on molecular level using 16S RNA. Then to test of influence of rhizobacterial isolates on cumin seed germination and seed vigour, a laboratory experiment was designed.

Cumin seeds were treated with different treatment such, T1 (DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162), T2 (DCU-181 *Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284), T3 (DCU-184 *Pseudomonas aeruginosa* NRCSSDCU184 Accession no. MN192163), T4 (DCU-188 *Pseudomonas aeruginosa* NRCSSDCU188 Accession no. MN192164), T5 (DCU-251*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165), T6 (DCU-262 *Kosakonia oryzendophytica* strain NRCSSDCU262 Accession no. MN192166), T7 (DCU-351*Pseudomonas aeruginosa* strain NRCSSDCU351 Accession no. MN192167), T8 (DCU-364 not identified), T9 (DCU-451 *Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), T10 (DCU-453 *Pseudomonas aeruginosa* NRCSSDCU453 Accession no. MN192169), T11 (DCU-651 *Bacillus pacificus strain* NRCSSDCU651 Accession no. MN192170) and T12 (Control) The number of treatments was twelve, replicated thrice in completely randomized design. Cumin (GC-4)) was taken as a test variety.

Observations recorded

First germination count (%): First count was observed on 6th day of the germination test based on the number of seeds started germination processes.

Germination (%): The laboratory germination test was conducted as per ISTA procedure by adopting top of the paper method. 20 seeds were treated with rhizobacterial isolates in three replications are taken then placed uniformly on germination paper. The top of the paper was kept in germinator, where the temperature was maintained at 25 ± 0.50ºC and the relative humidity at 95 ± 1 per cent. The final counts were made on fourteenth day of germination test for normal seedlings and germination was expressed in percentage.

Seedling length (cm): Ten normal seedlings were selected at random from the germination test. The length between the collar region and the tip of the primary shoot was measured as shoot length (cm). The length between the collar region and the tip of primary root was measured as root length (cm). The total seedling length was computed by using the following formula,

Seedling length (cm) = Shoot length (cm) + Root length (cm)

Seedling fresh weight (mg): To record seedling fresh weight ten seedling were counted, cut free from their cotyledon and weighted while still moist. Their weights were recorded in milligram.

Seedling dry weight (mg): The ten normal seedlings selected randomly from the germination test. These seedlings were kept in hot air oven for 24 hours at the temperature of 80ºC. The weight of the dried seedlings was recorded and dry weight of seedling was calculated and expressed in milligram.

Seedling Vigour index I: The vigour index I was calculated using the procedure suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Vigour index-I = Germination (%) X Seedling length (cm)

Seedling Vigour index II: The vigour index II was calculated using the procedure suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Vigour index-II = Germination (%) X Seedling dry weight (mg).

Statistical analysis

Experiment was arranged in completely randomized design. All the recorded observations were tabulated in a systematic manner. Values were given as means for their respective number of replications used. The data were subjected to Analysis of Variance (ANOVA) using the online statistical analysis (OPSTAT, Computer section, CCS HAU Hisar, Haryana).

Results and Discussion

Results of seed germination parameters *viz*. days taken to initiate seed germination, germination percentage and rate of seed germination were depicted in Table 1 (Fig. 1, Fig. 2). The minimum days taken to initiate seed germination was 6.33 recorded due to seed treatment with isolate DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) and the maximum days taken to initiate seed germination was 10.33 recorded in Control. Seed germination percentage were found increased significantly from 71.67 to 86.66 due to seed treatment over control. The highest germination percentage was recorded 86.66 followed by 83.33, 81.66 and 80 associated with DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-184 (*Pseudomonas aeruginosa* NRCSSDCU184 Accession no. MN192163), DCU-188 (*Pseudomonas aeruginosa* NRCSSDCU188 Accession no. MN192164) and DCU-262(*Kosakonia oryzendophytica* strain NRCSSDCU262 Accession no. MN192166), respectively. Germination percentage recorded in DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162 (73.33) and DCU-364 (75) were at par as compared to control. The lowest germination percentage was 71.67 recorded in control. Rate of seed germination were increased significantly from 0.95 (control) to 2.11 due to all treatment except DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162 and DCU-453. The highest rate of seed germination was 2.11 followed by 2.01, 2 and 1.79 associated with treatments DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-184 (*Pseudomonas aeruginosa* NRCSSDCU184 Accession no. MN192163), DCU-251 DCU-251(*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165) and DCU-181 (*Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284), respectively. Rate of germination recorded in DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162 (1.32) and DCU-453 (1.41) were at par. The lowest rate of germination was 0.95 recorded in control. Similar results by Ashrafuzzaman *et al.* (2009) reported that seed germination and seedling vigour of *Jatropha curcas* were increased due to plant growth promoting rhizobacterial isolates *Brevibacillus brevis* MS1, *Enterobacter aerogenes* MS2, *Bacillus licheniformis* MS3, *Micrococcus sp.* MS4 and *Acienetobacter calcoaceticus* MS5 over control. Similarly, Kumar *et al.* (2013) [2] evaluated isolates *Bacillus spp*., *Kocuria sp.*, *Anabaena laxa* and *Calothrix elenkinii* as plant growth promoting (PGP) agents with seed spices i.e. coriander, cumin and fennel, under controlled conditions. They reported that amendment with bacterial isolates brought about 25% enhanced germination in cumin over control.

Table 1: Effect of rhizobactterial isoates on germination of cumin

S.N.	Treatments	Treatments details	Initiation of seed germination (Days)	Germination percentage	Rate of Germination
1.	T1	DCU-22	8	73.33	1.32
2.	T2	DCU-181	7.33	78.33	1.79
3.	T3	DCU-184	7	83.33	2.01
4.	T4	DCU-188	6.66	81.66	1.78
5.	T5	DCU-251	8.33	76.66	2
6.	T6	DCU-262	8.66	80	1.74
7.	T7	DCU-351	7.33	78.33	1.62
8.	T8	DCU-364	7.33	75	1.75
9.	T9	DCU-451	6.33	86.66	2.11
10.	T10	DCU-453	7.66	76.14	1.69
11.	T11	DCU-651	9.33	76.66	1.41
12.	T12	Control	10.33	71.67	0.95
	SEm		0.683	2.033	0.246
	CD (5%)		1.442	4.125	0.516
	CV		4.962	3.375	5.496

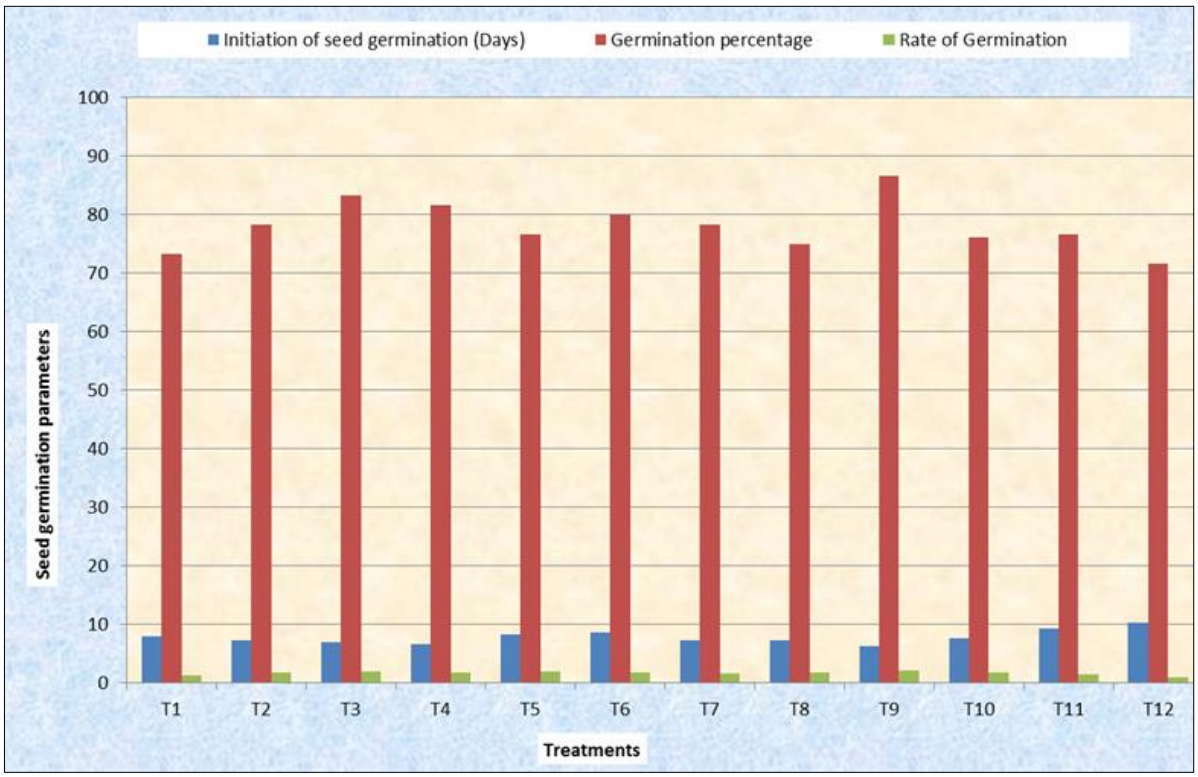

Fig. 1: Effect of rhizobacterial isoates on seed germination of cumin

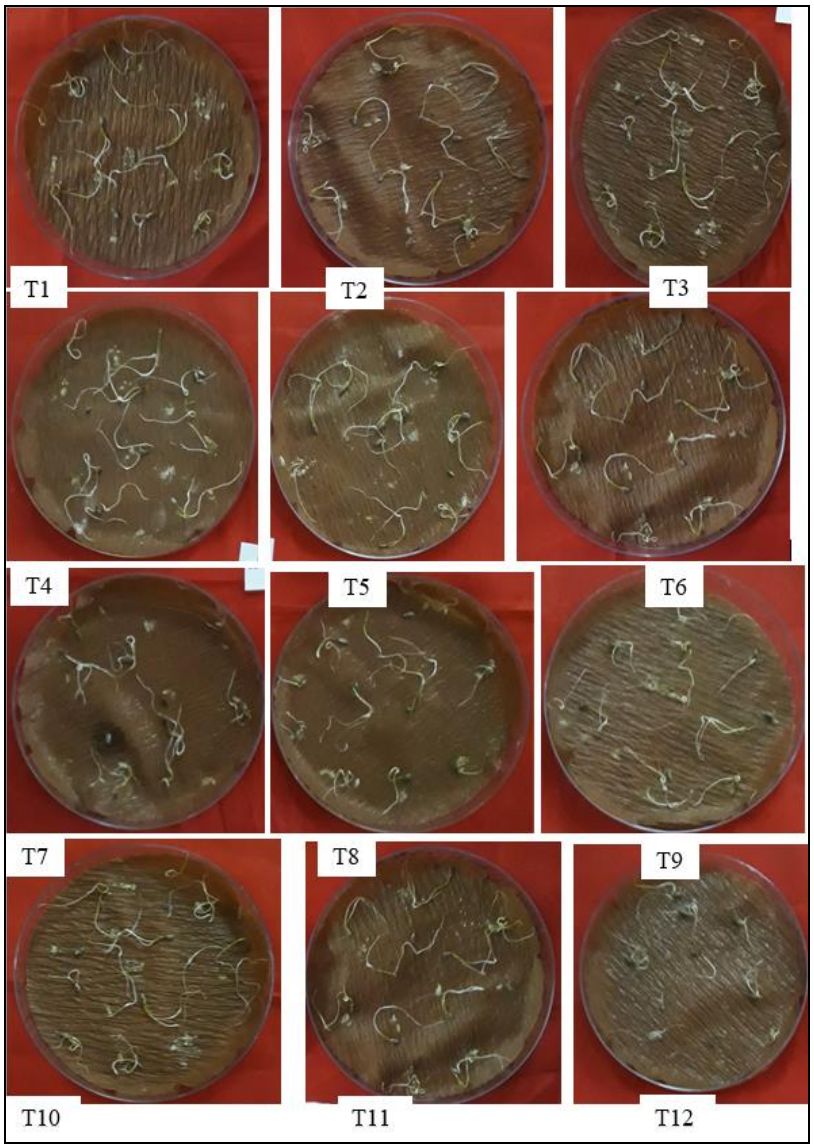

Fig 2: Effect of rhizobacterial isolates on Germination percentage in cumin @ 10 Days

(Fig. 3) clearly revealed that seedling dry weight and vigour index-I were found increased significantly due to all treatments over control while seedling length, seedling fresh weight and vigour index-II were increased significantly due to some treatment over control. Seedling length increased significantly from 5.16 (control) to 7.93, 7.83, 7.76, 7.63, 7.13, 6.76, 6.63, 6.50 and 6.06 cm due to seed treatment with isolates, DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-188, DCU-262, DCU-351, DCU-22, DCU-181, DCU-453, DCU-1651 and DCU-364, respectively. Value of seedling length associated with DCU-184 and DCU-251 were recorded at par as compared to control. The maximum seedling length was 7.93 followed by 7.83, 7.76 and 7.63 cm per plant, associated with isolates DCU-451, DCU-188, DCU-262 and DCU-351, respectively and minimum seedling length was 5.16 cm associated with control. Seedling fresh weight increased significantly from 108.43 to 145.18 mg due to all treatments except DCU-181 and DCU-251 over control. The highest seedling fresh weight was 145.18 followed by 139.8, 132.83 and 132.46 mg due to isolates DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-188, DCU-351 and DCU-262, respectively. Seedling fresh weight associated with DCU-181 (114.1 mg) and DCU-251 (112.13 mg) were recorded at par as compared to control. The lowest seedling fresh weight was 108.43 mg recorded in control. Seedling dry weight increased significantly from 9.55 to 14.03 mg due to all treatments over control. The highest seedling dry weight was 14.03 mg followed by 13.14, 12.5, 12.26, 11.12, 11.10, 10.91, 10.55, 10.37, 10.35 and 10.26 mg due to isolates DCU-451, DCU-188, DCU-351, DCU-453, DCU-184, DCU-651, DCU-181, DCU-364, DCU-251, DCU-262 and DCU-22, respectively. The highest seedling dry weight was 14.03 mg associated with DCU-451. The lowest seedling dry weight was 9.55 mg recorded in control.

Vigour index-I (Table 2, Fig. 3) increased significantly from 369.81 and 687.5 due to all treatments over control. The highest value of vigour index-I was 687.5 followed by 639.66, 620.66, 598.83, 530.33 and 523.16 due to seed treatment with isolates DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-188 (*Pseudomonas aeruginosa* NRCSSDCU188 Accession no. MN192164), DCU-262 (*Kosakonia oryzendophytica* strain NRCSSDCU262 Accession no. MN192166), DCU-351 (*Pseudomonas aeruginosa* strain NRCSSDCU351 Accession no. MN192167), DCU-181 (*Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284) and DCU-22 (*Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162), respectively. The lowest value of vigour index-I

was 369.81 associated with control. Vigour index-II (Table 2, Fig. 3) increased significantly from 684.44 to 1211.16 due to all treatments over control except DCU-22 (*Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162). The highest value of vigour index-II was 1211.16 followed by 1074.13, 984.16, 933.47, 926.62, 854.56, 847.66 and 837.46 due to seed treatment with isolate DCU-451 (*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168), DCU-188 (*Pseudomonas aeruginosa* NRCSSDCU188 Accession no. MN192164), DCU-351 (*Pseudomonas aeruginosa* strain NRCSSDCU351 Accession no. MN192167), DCU-453, DCU-184, DCU-181 (*Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284), DCU-651(*Bacillus pacificus strain* NRCSSDCU651 Accession no. MN192170) and DCU-262 (*Kosakonia oryzendophytica* strain NRCSSDCU262 Accession no. MN192166), respectively. Vigour index-II associated with DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162 (754.23) was at par. The lowest vigour index-II was 684.44 recorded in control. Similar findings were reported by Mishra *et al.* (2017) [4] in coriander in which they observed that highest seedling vigour index was recorded for *B. aerophilus* Cor-15 (1178.50) followed by *B. megaterium* (1125.20) and minimum was observed with control. Similarly Kumar *et al.* (2013) [2] reported that the plant response to microbial inoculation, measured as vigour index showed highest values in fennel crop (1200–1300), with 40–80% increase over controls (no inoculation) was recorded.

Table 2: Effect of rhizobacterial isolates on seedling length, seedling biomass and vigour index of cumin

Treatments	Treatments details	Seedling length (cm)	Seedling fresh wt. (mg)	Seedling dry wt. (mg)	Seedling vigour Index-I	Seedling vigour Index-II
T1	DCU-22	7.13	125.13	10.26	523.16	754.23
T2	DCU-181	6.76	114.1	10.91	530.33	854.56
T3	DCU-184	5.86	116.13	11.12	488	926.62
T4	DCU-188	7.83	139.8	13.14	639.66	1074.13
T5	DCU-251	5.87	112.13	10.37	450.43	795
T6	DCU-262	7.76	132.46	10.33	620.66	837.46
T7	DCU-351	7.63	132.83	12.5	598.83	984.16
T8	DCU-364	6.06	119.8	10.55	454.33	793.38
T9	DCU-451	7.93	145.18	14.03	687.5	1211.16
T10	DCU-453	6.63	116.46	12.26	504.80	933.47
T11	DCU-651	6.5	117.16	11.1	498.33	847.66
T12	Control	5.16	108.43	9.55	369.81	684.44
	SEm	0.391	3.719	0.847	26.935	28.793
	CD(5%)	0.823	7.502	1.913	64.366	77.931
	CV	3.649	5.178	4.973	8.496	11.206

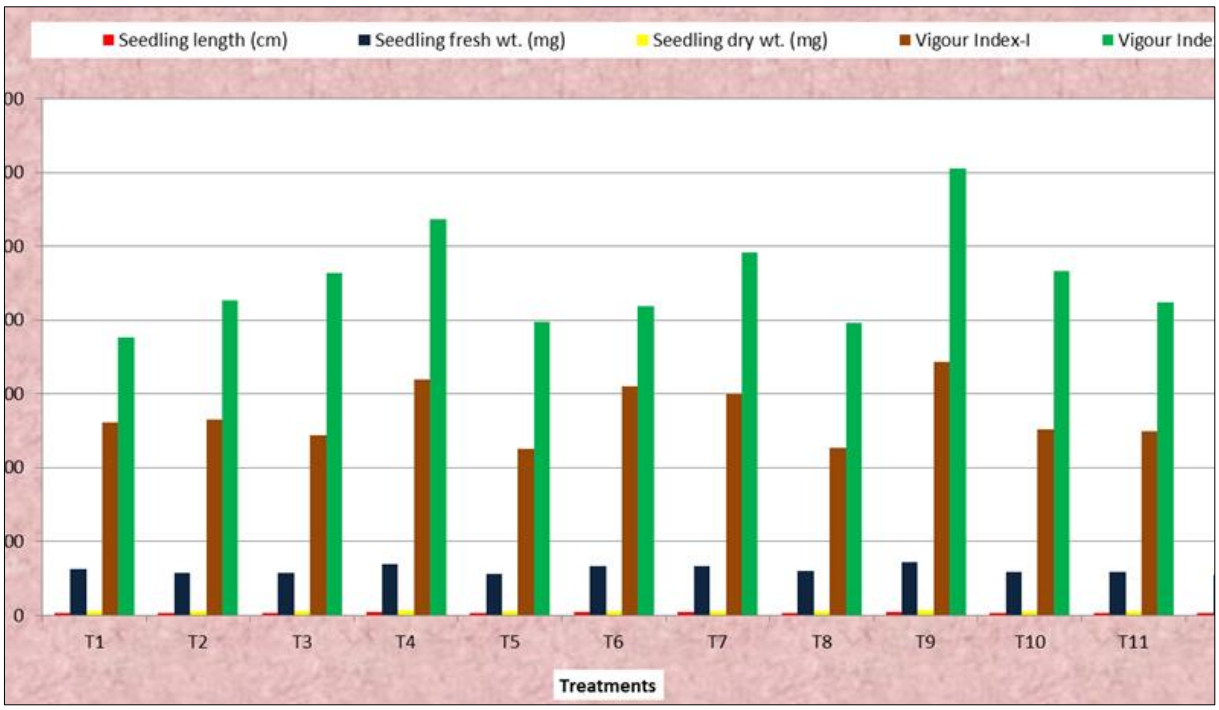

Fig. 3: Effect of rhizobacterial isolates on length, biomass and vigour index of cumin seedling

Conclusion

The overall improvement in seedling vigour through a significant increase in various physiological parameters suggests that these strains have a plant-growth promoting ability on cumin seedlings and hence could be used for seed inoculation for better establishment of seedlings. The plants with enhanced seedling vigour can help in better establishment of plants. All the eleven isolates DCU-22,

DCU-181, DCU-184, DCU-188, DCU-251, DCU-262, DCU-351, DCU-364, DCU-451, DCU-453 and DCU-651, considering the plant growth promoting abilities of these eleven isolates for bioinoculant preparation is possible. DCU-451 *Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168 was superior. This study show that these isolates having best characteristics of plant growth promoting potential tha help in the seed germination and Seed vigour parameters of cumin plant.

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