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In vitro Bio control Efficiency of Bacterial Endophytes and its Effect on Growth Parameter of Chilli (*Capsicum annum* L.)

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ABSTRACT: The antagonistic activity of endophytic bacteria against two phytopathogens *viz*., *Fusarium oxysporum*, *Colletotrichum* sp. were isolated from healthy tissues of chilli and tomato plants in 2018-2019 and the pot experiment was carried out in the glass house of Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur with eighteen treatments and three replications. To explore the antifungal activity was screened using dual culture method in which both endophytic bacteria and test fungi were inoculated in single Potato Dextrose Agar (PDA) media plate. The potential endophytic bacterial isolates were identified through morphological and biochemical characteristics revealed that the *Bacillus* species were the dominant antagonist. The results of present study indicated that the isolate ESK-26 and ESK-32 recorded highest per cent inhibition of 63.85 % and 61.25 % against *Fusarium oxysporum* and *Colletotrichum* spp. respectively. Significantly highest plant height, number of leaves per plant and total dry weight of chilli plants were recorded by the consortium of endophytic bacteria (ESK-26 + ESR-6) at 60 and 90 DAS as compared to control. Hence, these isolates are promising plant growth promoting isolates showing multiple attributes that can significantly influence the chilli growth and provide a strong basis for further development of bio-inoculants to attain the desired plant growth promoting activity in chilli growing fields.

Keywords: Endophytic bacteria, *Bacillus* sp. Plant growth promotion, chilli, *Fusarium oxysporum* and *Colletotrichum* spp.

INTRODUCTION

The term "endophyte" is derived from the Greek words "endon" meaning within, and "phyton" meaning plant. Endophytic microbes, residing in plant play a powerful role in exerting their beneficial attributes with a higher consistency, particularly they dwell in a relatively secure environment, largely protected from the externally induced abiotic or biotic stresses (Shatrupa *et al.*, 2018). All plant taxa investigated have well established symbiosis relations with a large variety of microorganisms. These microorganisms are known to support plant growth and to increase plant tolerance to biotic and abiotic stresses (Ali *et al*., 2018). Nearly 300000 plant species that exist on the earth, each individual plant is host to one or more endophytes (Strobel *et al*., 1993). Endophytes need to initially enter the plant endosphere, adapt new environment and are able to penetrate and become systematically disseminated in the host plant. Actively colonizing the apoplast, conducting vessels and occasionally the intracellular spaces (Hallmann *et al.*, 1997). Root endophytes often colonize and penetrate the epidermis at sites of lateral root emergence, root hair zone and root cracks and residing within apoplastic space between plant cells (Schulz and Boyle, 2006). Cellulase and protease act as key enzymes for the invasion and colonization of plant roots (Susilowatim *et al.*, 2015).

Endophytic bacteria have been isolated from a large diversity of plants. Organisms like *Bacillus, Enterobacter, Klebsiella, Pseudomonas, Burkholderia, Pantoea, Agrobacterium, Methylobacterium* spp. Constitute the endophytes commonly isolated from diverse plants such as rice, wheat, maize, cotton, clover, potato, sugarcane, tomato, cucumber, and wild grasses (Bacon and Hinton, 2006). The precise role of endophytes in plants is not yet known. However, their capability to thrive within the host tissues away from microbial competition and environmental degradation has made endophytes potential candidates for use in agriculture.

Microbial populations are key component of soil plant system where they are immense in a network of interaction s affecting plant development (Kumar *et al*., 2012). Use of endophytic bacteria presents a special interest for development of agricultural applications that ensure improved crop performance under cold, drought or contaminated soil stress conditions or enhanced disease resistance.

In Karnataka, Chilli (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum* Mill.) are two major transplanted crops, which belong to the family solanaceae which has 90 genera and 2000 species. It is used in both green and dry forms as an ingredient in preparation of several spicy dishes. Chilli is native of Central America and is a well known spice crop due to the presence of 'Capsaicin' which imparts potency. It appears pink after ripening due to the pigment 'Capsanthin'. It is a rich source of ascorbic acid. Chilli is grown over an area of 364 thousand hectares with the production of 37 million tonnes in India and the major chilli growing states in India are Andhra Pradesh, Karnataka, Tamil Nadu, Madhya Pradesh and West Bengal. Among them, in Karnataka, it is grown in 168 thousand hectares area with 52 thousand tonnes of yield (Anonymus, 2020). This study was conducted to isolate and evaluate bacterial endophytes for their plant growth promotion attribute.

MATERIAL AND METHODS

A. Isolation of endophytic microorganisms from leaf, stem and roots of chilli

The isolation of endophytes was carried out as per the standard procedures given by Bacon *et al*. (2002). The randomly selected plants were uprooted manually and washed in running tap water. Stem sections of 2 cm length were excised using flame sterilized scalpel from 1 cm to 2 cm above the soil line. The leaf and root sections were similarly prepared. All the samples were blotted dry with filter paper and then weighed to have final sample of 0.5 gm. The surface sterilization of the stem, root and leaf pieces were carried out with the following immersion sequence: 70 per cent ethanol for 1 min, 3 per cent sodium hypochlorite for 5 min in young plants and 10 min in case of older plants followed by 70 per cent ethanol wash for 1 min. They were then rinsed four times with sterile water and dried in laminar air flow. Surface disinfestations parameters like selection of disinfectant, its strength, duration of immersion in disinfectant were optimized prior to experimentation. The cut ends of surface sterilised segments were removed with flame sterilized scalpel and were placed in appropriate agar media with the cut surface touching the agar. The plates were incubated for four to eight days at 28 °C and liquid from plant sample oozes from cut ends due to osmotic pressure. The oozed liquid contains endophytes which forms growth on agar plate on edged of plant sample touching agar. The plates were observed for the presence of bacterial endophytes.

B. Biocontrol efficiency of endophytic bacterial isolates against fungal pathogens (Dennis and Webster, 1971)

Endophytic bacterial isolates were tested for their *in vitro* antagonist activity against two major pathogens of vegetables. The phytopathogenic fungi were the root pathogens *Fusarium oxysporum*, and the leaf pathogen *Colletotrichum* sp. They were collected from the Dept. of Plant Pathology, College of Agriculture, Raichur, Karnataka.

Antifungal activity was screened using dual culture method in which both endophytic bacteria and test fungi were inoculated in single Potato Dextrose Agar (PDA) media plate. Loopful of 24 h old culture of endophytic bacterial isolate was streaked on the potato dextrose agar plate at one end, which was pre-inoculated with 5 days old, 5 mm mycelial disc of test pathogen at the other end. The control plate was maintained by placing only pathogen mycelial disc in the centre without bacteria. The assay plates were incubated at 28 ± 1 °C for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate, three replications were maintained with suitable controls. The per cent growth inhibition over control was calculated by using the formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition,

C = Growth of fungal plant pathogens in control (mm),

T = Growth of fungal plant pathogens in dual culture plate (mm)

Further, based on characterisation and screening five efficient isolates *viz.,* ESR-6 (*Pseudomonas* sp.), ESY-15 (*Bacillus* sp.), ESK-26 (*Bacillus* sp.), ESK-32 (*Bacillus* sp.) and ESB-44 (*Acinetobacter* sp.) were selected for pot experiment.

Different growth parameters (plant height, number of leaves per plant, number of branches per plant and total dry matter production) were set to evaluate the efficiency of endophytic bacterial isolates. The observations were recorded at different intervals like 60 Days After Sowing (DAS) and 90 DAS of the crop.

Pot culture experiment. The pot culture experiment was conducted in the glass house of Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur during 2019 to study the effect of endophytic isolates on growth parameters of chilli

Details of pot experiment. The treatment was laid out in Completely Randomized Design (CRD) with three replications. The treatment details were furnished in Table 1.

Table 1: Experimental details of pot experiment.

Sr.No.	Particulars	Details
1	Treatments	T_1 - Uninoculated control
		T_2 - 100 % RDF
		T_3 - 75 % RDF + Efficient isolate of endophytic bacteria (ESK-26)
		T_4 - 75 % RDF + Efficient isolate of endophytic bacteria (ESR-6)
		T_5 - 75 % RDF + Efficient isolate of endophytic bacteria (ESK-32)
		T_6 - 75 % RDF + Efficient isolate of endophytic bacteria (ESY-15)
		T_7 - 75 % RDF + Efficient isolate of endophytic bacteria (ESB-44)
		T_8 - 75 % RDF + (ESK-26) + (ESR-6)
		T_9 - 75 % RDF + (ESK-26) + (ESK-32)
		T_{10} - 75 % RDF + (ESK-26) + (ESY-15)
		T_{11} - 75 % RDF + (ESK-26) + (ESB-44)
		T_{12} - 75 % RDF + (ESR-6) + (ESK-32)
		T_{13} - 75 % RDF + (ESR-6) + (ESY-15)
		T_{14} - 75 % RDF + (ESR-6) + (ESB-44)
		T_{15} - 75 % RDF + (ESK-32) + (ESY-15)
		T_{16} - 75 % RDF + (ESK-32) + (ESB-44)
		T_{17} - 75 % RDF + (ESY-15) + (ESB-44)
		T_{18} - 75 % RDF + Reference strain
2	Crop	Chilli
3	Design	Completely Randomized Design (CRD)
4	Replication	3
5	Treatment	18

Preparation of pots. The potting mixture in all the experiments consisted of soil: farmyard manure in 2:1 proportion was sieved through a 4 mm mesh and then autoclaved for 3 h at 121°C in autoclavable plastic bags. The soil having physiochemical properties of pH 7.2, Electrical conductivity 0.26 dS/m, organic carbon 0.93 per cent, available nitrogen 290.6 kg/ha, available

phosphorus 11.24 kg/ha and available potassium 106.00 kg/ha were also analyzed before filling the pots. Each earthen pot was filled with the 10 kg of sterilized soil.

Bacterial culture preparation for seed inoculation. The endophytic isolates *viz.*, ESK-26, ESR-6, ESK-32, ESY-15 and ESB-44 and consortium of the above five was used for this study. A loopful of the endophytic isolates were inoculated into the nutrient broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 °C). After 48 h of incubation, ten milliliter of the broth containing a population of 10^8 cfu/ml was used for inoculation. The bacterial strains were grown separately and the five strains that are going to make up the combination were added equally (v/v) and finally mixed at the time of inoculation.

Seed bacterization. Chilli seeds were surface sterilized with 70 per cent ethanol for 1 minutes, 3 per cent sodium hypochlorite for 5 minutes followed by 70 per cent ethanol wash for 1 min, rinsed in sterile distilled water thrice and dried overnight under sterile air stream. Required quantity of seeds were soaked in ten ml of bacterial suspension containing 10^8 cfu/ml for 12 h and dried under laminar air flow. The seeds soaked in sterile distilled water were maintained as control. Seeds were sown in respectively labelled pots at a depth of 5-6 cm followed by water application was carried out to moisten the seeds to encourage germination.

Phyllosphere Spray (PS). The phyllosphere spray was given at the flowering stage of the crop. The standard inoculum of the test endophytic isolate combination was diluted at 1:1 ratio with sterile water and sprayed on the leaf at early morning or evening to have uniform wetting.

Observations recorded for chilli. Observations on plant growth parameters were recorded at 30, 60 and 90 DAS of the chilli crop.

Height of the plant from base to the tip of the main stem was recorded in centimetres and the mean value was calculated. The total number of leaves in the plant was counted and the mean was calculated and expressed as number. Total number of branches on each plant was counted and the average number of branches were calculated. The shoots and roots were first washed and then dried in shade for 24 to 36 h. Then they were dried in hot air oven at 50 °C until constant dry weight was obtained and the average dry weight of the plant was expressed in grams.

RESULT AND DISCUSSION

Isolation of endophytic bacteria. The bacterial endophytes were isolated from root, stem and leaves of chilli plants which are collected from north eastern zones of Karnataka. Out of 50 plant samples, 48 isolates were obtained and purified by streak plate technique.

Biocontrol efficiency of endophytic bacteria. A total of 48 endophytic bacterial isolates were tested for their antagonistic activity under *in vitro* against *Fusarium oxysporum* and *Colletotrichum* spp. by dual culture technique. Among the isolates, 14 and 11 isolates were showed positive for antagonistic activity against *F. oxysporum* and *Colletotrichum* spp. respectively, the results obtained were presented in Table 2.

Table 2. *In vitro* screening of endophytic bacterial isolates against *Fusarium oxysporium and Colletotrichum* sp. by dual culture method.

Sr. No.	ISOLATE	*Fusarium oxysporium*		*Colletotrichum* sp.	
		Radial growth (mm)	Inhibition of pathogen (%)	Radial growth (mm)	Inhibition of pathogen (%)
1.	ESR-3	46.58	40.28^{g}	-	-
2.	ESR-5	38.74	50.33^{cd}	46.84	40.33^{e}
3.	ESR-6	32.12	58.82^{b}	39.12	50.17^{c}
4.	ESR-8	39.36	49.54^{cde}	48.25	38.54^{e}
5.	ESR-12	-	-	-	-
6.	ESY-14	-	-	-	-
7.	ESY-15	37.11	52.42^{c}	43.36	44.76^{d}
8.	ESY-17	41.37	46.96^{ef}	48.08	38.75^{e}
9.	ESY-23	38.86	50.18^{cd}	-	-
10.	ESY-24	-	-	42.14	46.32^{d}
11.	ESK-26	28.20	63.85^{a}	32.70	58.34^{b}
12.	ESK-28	-	-	-	-
13.	ESK-32	39.20	49.74^{cde}	30.42	61.25^{a}
14.	ESK-33	40.86	47.62^{def}	48.12	38.70^{e}
15.	ESK-34	-	-	-	-
16.	ESK-35	42.23	45.86^{f}	-	-
17.	ESK-36	46.92	39.85^{g}	-	-
18.	ESK-39	40.91	47.55^{def}	-	-
19.	ESB-44	38.38	50.80^{c}	42.22	46.22^{d}
20.	ESB-47	-	-	48.84	37.78^{e}
21.	Control	78.00	0.00^{h}	78.50	0.00^{f}
	S.Em±		1.01		0.95
	CD at 5 %		3.02		2.84

***Fusarium oxysporum*.** Among the isolates, only six isolates were showed more than 50 per cent inhibition of pathogen growth. The isolate ESK-26 recorded the highest per cent inhibition of 63.85 per cent followed by ESR-6 (58.82 %) and ESY-15 (52.42 %). Lowest per cent inhibition was recorded with ESK-36 (39.85 %).

These results were in agreement with the findings of Khan *et al.* (2020) who reported that endophytic bacterial strain SK1 (*Paenibacillus polymyxa*) showed the high capability to inhibit mycelial growth of the test pathogens *Fusarium oxysporum* (55.54 %). The strain SK1 exhibited considerable inhibition potential against all the tested pathogenic strains, might be due to the release of diffusible compounds against the test pathogens. Similarly, Suryanto *et al.* (2018) reported that five different endophytic bacteria *viz.*, SDW1, SDW2, SDW3, SDW4 and SDW5 have been isolated from chilli plant. The isolate SDW2 showed more to inhibition followed by SDW1 against *Fusarium oxysporum*. Similarly, Antagonistic activity was recorded by Egamberdieva *et al*. (2017) for endophytes against plant pathogenic fungi such as *Fusarium oxysporum, F. solani, F. culmorum,*

Alternaria alternata, and *Botrytis cinerea*. The isolate EB2 was highly effective against *Fusarium* pathogens, and only isolate EB10 showed antagonistic activity against all of the fungal pathogens. Siderophore producing endophytic bacteria stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria, that suppresses the growth of pathogenic organisms *viz.*, *Fusarium oxysporum and Rhizoctonia solani*, function as stress factors in inducing host resistance (Haas and Defago 2005; Joseph *et al.*,2007; Wahyudi *et al.*, 2011).

***Colletotrichum* spp.** The isolate ESK-32 recorded highest per cent inhibition (61.25 %) on *Colletotrichum* spp. under *in vitro* condition followed by ESK-26 (58.34 %) and ESR-6 (50.17 %). The least per cent inhibition (37.78 %) was observed with ESB-47.

Similar results were obtained by Paul *et al.* (2013) Endophytic bacteria were evaluated for antagonistic activity against five phytopathogenic fungi. Twenty two endophytic bacteria were active against at least one tested fungi. The percentage of endophytic bacteria showed strong pathogenic fungal inhibition were 3.3 per cent, 2.7 per cent against *Colletotrichum acutatum*, *Fusarium oxysporum*, Species of *Bacillus*, *Paenibacillus*, *Burkholderia*, *Pseudomonas* showed strong and broad spectrum antifungal activity against all pathogenic fungi. It was due to production of lytic enzyme, might be due to siderophore production by the endophytic bacteria. Similarly, the antagonistic potential of endophytic bacteria isolated by Amaresan *et al*. (2014) from chilli plants were determined *in vitro* against four pathogens, *viz*., *Sclerotium rolfsii*, *Fusarium oxysporum*, *Colletotrichum capsici* and *Pythium* sp. The effect of endophytic bacteria towards these fungi revealed that most of the isolates showed antagonistic activity against *Pythium* sp. (37.8 %), followed by 35.1 per cent isolates against *F. oxysporum* and *C. capsici*, and 21.6 per cent to *S. rolfsii*.

Growth parameter. The inoculation effect of endophytic bacteria with 75 % RDF on growth parameters of chilli plants were studied under pot culture conditions using five efficient strains of endophytic bacteria (ESK-26, ESR-6, ESK-32, ESY-15 and ESB-44) selected on the basis of biochemical characterisation, molecular characterisation and production of growth promoting substances. The data pertaining to plant height, number of leaves per plant, number of branches per plant, and total dry weight of chilli plant at various stages of crop growth was influenced by bacterial inoculation of endophytes were presented in Table 3 & 4.

Table 3: Influence of endophytic bacterial isolates on plant height and number of leaves per plant at different growth stages of chilli under pot experiment.

Treatment	Plant height of chilli (cm)		Number of leaves per plant	
	60 DAS	90 DAS	60 DAS	90 DAS
T_1 - Uninoculated control	15.15^{j}	24.18^{i}	18.92^{j}	42.70^{m}
T_2 - 100 % RDF	19.76^{i}	38.14^{h}	25.54^{i}	53.56^{l}
T_3 - 75% RDF + (ESK-26)	24.64^{fg}	43.24^{ef}	28.76^{fgh}	59.70^{fgh}
T_4 - 75% RDF + (ESR-6)	22.74^{h}	42.50^{f}	27.6^{h}	57.06^{ij}
T_5 - 75% RDF + (ESK-32)	20.98^{i}	40.70^{g}	26.42^{i}	55.44^{ik}
T_6 - 75% RDF + (ESY-15)	20.06^{i}	39.86^{g}	25.87^{i}	54.18^{kl}
T_7 - 75% RDF + (ESB-44)	19.86^{i}	39.56^{gh}	25.62^{i}	53.83^{kl}
T_8 - 75% RDF + (ESK-26) + (ESR-6)	32.52^{a}	53.54^{a}	36.24^{a}	68.72^{a}
T_9 - 75% RDF + (ESK-26) + (ESK-32)	30.04^{b}	50.75^{b}	34.48^{b}	66.14^{b}
T_{10} - 75% RDF + (ESK-26) + (ESY-15)	27.56^{cd}	46.28^{d}	32.6^{c}	64.72^{bc}
T_{11} - 75% RDF + (ESK-26) + (ESB-44)	26.92^{cde}	45.07^{d}	30.13^{de}	62.76^{de}
T_{12} - 75% RDF + (ESR-6) + (ESK-32)	28.00^{c}	48.02^{c}	33.78^{b}	65.68^{b}
T_{13} - 75% RDF + (ESR-6) + (ESY-15)	27.17^{cde}	45.73^{d}	30.96^{d}	63.13^{cd}
T_{14} - 75% RDF + (ESR-6) + (ESB-44)	26.08^{def}	44.84^{de}	29.74^{ef}	61.25^{ef}
T_{15} - 75% RDF + (ESK-32) + (ESY-15)	25.83^{ef}	44.77^{de}	29.62^{ef}	60.60^{fg}
T_{16} - 75% RDF + (ESK-32) + (ESB-44)	25.13^{f}	43.33^{ef}	28.92^{fg}	60.05^{fgh}
T_{17} - 75% RDF + (ESY-15) + (ESB-44)	23.17^{gh}	42.63^{f}	27.95^{h}	58.38^{hi}
T_{18} - 75% RDF + Reference strain	23.33^{gh}	42.82^{f}	28.03^{gh}	59.17^{gh}
S.Em ±	0.52	0.57	0.39	0.56
CD at 5%	1.56	1.71	1.17	1.67

DAS: Days After Sowing;

*Mean values followed by the different letter are significantly different (P<0.05) from each other as evaluated from Duncan's multiple range test (DMRT).

The result indicated that the treatment T_8 inoculated with endophytic bacterial consortia recorded highest plant height (32.52 cm and 53.54 cm) and number of leaves per plant (36.24 and 68.72) at 60 and 90 DAS respectively, which was significantly superior over all other treatments. The present results were in line with Khan *et al*. (2020) reported that, inoculation of endophytic bacterial strain *P. polymyxa* SK1 had a positive correlation in terms of increased plant height. It was due to the isolated strain SK1 showed plant growth-promoting traits such as the production of organic acids, ACC deaminase, IAA, siderophores, nitrogen fixation, and phosphate solubilization. Similar outcomes were noticed with Rohini *et al*. (2018) observed that, inoculation of endophytic bacterial strain ZoB19 (*Enterobacter cloacae*) had profuse effect on leaf number in *Vigna unguiculata*. It was due to balanced regulation on various PGP traits such as phosphate solubilization, 1-amino cyclopropane 1-carboxylate (ACC) deaminase activity, nitrogen fixation, and ammonia and IAA production. The result obtained in the present study indicated that, significantly highest number of branches per plant (4.38 and 6.24) and total dry weight (11.83 g/plant and 20.98 g/plant) of chilli plant was recorded at 60 and 90 DAS respectively. A similar observations were also made by Suleman *et al.* (2018) reported that wheat inoculation with selected endophytic strain MS16 showed pronounced effect on number of tillers in field trials. It was due to phosphate solubilization activity, indole-3-acetic acid, gibberellic acid, solubilized zinc compounds and showed nitrogenase and 1- Aminocyclopropane-1-carboxylic acid deaminase activity. Similar observation were noticed by Safdarpour and Khodakaramian (2019) who reported that, the increased tomato biomass was noticed by the inoculation of endophytic bacterial strains *P. mosselii*, *P. fluorescens*, *S. maltophilia* and *A. Calcoaceticus*. It was due to plant growth promoting bacteria (PGPB) that include the production of phytohormones such as indole-3-acetic acid (IAA), nitrogen fixation, phosphate solubilization and iron sequestration by bacterial siderophores.

Table 4: Influence of endophytic bacterial isolates on number of branches and total dry weight at different growth stages of chilli under pot experiment.

Treatment	Number of branches per plant		Total dry weight (g/plant)	
	60 DAS	60 DAS	60 DAS	90 DAS
T_1 - Uninoculated control	2.38^{m}	2.38^{m}	4.34^{n}	7.00^{m}
T_2 - 100 % RDF	2.80^{l}	2.80^{l}	5.45^{m}	9.53^{l}
T_3 - 75% RDF + (ESK-26)	3.12^{ghi}	3.12^{ghi}	6.70^{hi}	13.08^{h}
T_4 - 75% RDF + (ESR-6)	2.92^{jkl}	2.92^{jkl}	6.06^{kl}	10.89^{jk}
T_5 - 75% RDF + (ESK-32)	2.90^{kl}	2.90^{kl}	5.79^{klm}	10.57^{jk}
T_6 - 75% RDF + (ESY-15)	2.86^{l}	2.86^{l}	5.69^{lm}	10.09^{kl}
T_7 - 75% RDF + (ESB-44)	2.82^{l}	2.82^{l}	5.52^{m}	9.70^{l}
T_8 - 75% RDF + (ESK-26) + (ESR-6)	4.38^{a}	4.38^{a}	11.83^{a}	20.98^{a}
T_9 - 75% RDF + (ESK-26) + (ESK-32)	4.12^{b}	4.12^{b}	10.87^{b}	19.12^{b}
T_{10} - 75% RDF + (ESK-26) + (ESY-15)	3.71^{d}	3.71^{d}	9.10^{d}	17.56^{cd}
T_{11} - 75% RDF + (ESK-26) + (ESB-44)	3.40^{e}	3.40^{e}	8.44^{e}	16.50^{e}
T_{12} - 75% RDF + (ESR-6) + (ESK-32)	3.94^{c}	3.94^{c}	10.02^{c}	18.00^{c}
T_{13} - 75% RDF + (ESR-6) + (ESY-15)	3.56^{d}	3.56^{d}	8.60^{e}	16.92^{de}
T_{14} - 75% RDF + (ESR-6) + (ESB-44)	3.28^{ef}	3.28^{ef}	7.85^{f}	15.58^{f}
T_{15} - 75% RDF + (ESK-32) + (ESY-15)	$3.26e^{fg}$	$3.26e^{fg}$	7.48^{fg}	15.09^{fg}
T_{16} - 75% RDF + (ESK-32) + (ESB-44)	3.20^{fgh}	3.20^{fgh}	7.08^{gh}	14.29^{g}
T_{17} - 75% RDF + (ESY-15) + (ESB-44)	3.02^{ijk}	3.02^{ijk}	6.22^{ijk}	11.10^{ij}
T_{18} - 75% RDF + Reference strain	3.06^{hij}	3.06^{hij}	6.52^{ij}	11.91^{i}
S.Em ±	0.06	0.06	0.16	0.29
CD at 5%	0.16	0.16	0.46	0.86

DAS: Days After Sowing

*Mean values followed by the different letter are significantly different (P<0.05) from each other as evaluated from Duncan's multiple range test (DMRT).

CONCLUSION

In the present study, endophytic bacteria *viz.*, ESK-26, ESK-32 and ESR-6 showed highest antagonistic activity with plant growth promoting characteristics. The integrated use of these strains was demonstrated one of the sustainable options to reduce the usage of chemical fertilizers for sustainable agriculture. Future, *in vivo* assessment should aim to confirm the potential of these strains for use as bioinoculants in order to reduce chemical fertilizers and increase crop growth and productivity.

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