



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

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SCREENING OF *STREPTOMYCES DIASTATICUS* FOR *IN-VITRO* PLANT GROWTH PROMOTING TRAITS ON CHILLI (*CAPSICUM ANNUUM L.*)

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Received 16th July 2022; Revised 20th Sept. 2022; Accepted 23rd Jan. 2023; Available online 1st Oct. 2023

<https://doi.org/10.31032/IJBPAS/2023/12.10.7447>

ABSTRACT

The biological component known as Plant - growth Promoting Activity is thought to enhance plant growth. Actinomycetes is one category that plays a significant part in fostering plant growth. The present study showed the evaluation of plant growth promoting activity of *Streptomyces diastaticus* isolated from maize rhizosphere soil. Using Salkowsky's reagent and colorimetric quantification, a total of 18 isolates were screened for auxin production. The standard curve of IAA (Indole-3-acetic acid) was plotted against a wavelength range of 530 nm. The concentration of IAA produced by *S. diastaticus* was very high. The highest phosphate solubilizing efficiency was showed by *S. diastaticus*. Ammonia production was also found to be positive. The seed germination and plant growth was carried out *In-vitro* screening. Significant increase in shoot and root length was observed after 5 and 7 days with *S. diastaticus* compared to water treated control plants by Ragdoll method. The development and growth of Chilli were greatly enhanced by a strain of *Streptomyces* sp. having high plant growth promoting capabilities. As a result of the study, it can be concluded that the rhizospheric PGP *Streptomyces diastaticus* is an excellent agent to be developed as biofertilizers for growth promotion and yield enhancement in chilli crop and can be used for the industrial synthesis of a variety of agro-active chemicals.

Keywords: *Streptomyces* sp., Rhizosphere, Chilli, Plant growth promoting, IAA, Vigour index.

INTRODUCTION

Modern agricultural systems aim to ensure sustainable agricultural production, thus encouraging the search for new natural resources to find ecological solutions to protect plants and increase productivity [1]. Soil is an important part of the natural environment and is necessary to sustain life. Soil is made up of minerals and organic matter that stores nutrients and makes water available to plants [2]. Plant growth in soil depends on many biotic and abiotic factors. The thin layer of soil immediately surrounding plant roots is an important area for root function and metabolism and is called the rhizosphere. Many bacterial species, including actinomycetes, are associated with the rhizosphere and have been shown to be beneficial to plants [3]. A group of Gram-positive bacteria, the Rhizosphere Actinomycetes, have emerged as the most promising agent for biofertilizer formulations. Some genera of actinomycetes, including *Streptomyces*, have been widely developed to increase crop productivity. *Streptomyces* is the most studied genus for plant growth-promoting activity [2].

Phosphorus (P) is an essential macroelement for plants, yet the total concentration of P in soil ranges from 0.02 % to 0.5 %; an average

approximately 0.05%. The variation being largely due to differences in the weathering intensity and parent material composition. Thus, to increase the availability of phosphorus for plants, large amount of fertilizers are used on a regular basis, yet after application, a large proportion of the fertilizer phosphorus is quickly transferred to an insoluble form [4]. An appealing approach that has been intensively researched over the past ten years is the solubilization of phosphate-containing inorganic compounds by microbes. Actinomycetes are of particular importance among the Phosphate Solubilizing Microbes because they may grow as filamentous spores in a variety of soil types and create a variety of compounds (such as phytohormone-like compounds, insecticides, antihelminthics, and antifungals) that may aid plant growth [5]. Organic nitrogen in the soil can be converted into ammonia (NH₄⁺) by ammonifying bacteria, providing nitrogen to plants. Peptone water broth was used for qualitatively investigate ammonia production [6].

IAA is a common natural plant hormone with an indole ring and a microbial metabolite L-tryptophan. Several species of plant growth-promoting bacteria, including actinomycetes, enhance plant growth through the production

of IAA and an L-tryptophan-dependent mechanism. Some studies have shown that *Streptomyces* spp. an IAA-producing actinomycetes, is the dominant genus for IAA production. IAA play a role in many developmental and physiological processes in plants, including embryogenesis, organogenesis, root differentiation, root and shoot development, vegetative growth, and fruit development [7].

IAA synthesized by actinomycetes affects the root system by increasing the volume, weight, a number of lateral roots, and soil contact area. This mechanism improves plant development and yield by increasing the search and absorption of nutrients in the soil. IAA can also act as reciprocal signaling molecules, influencing gene expression in many bacteria, and also play an important role in plant-microbial interactions [8]. In addition to the production of plant hormones, *Streptomyces* spp. can also mobilize nutrients for host plants [9]. India is a large consumer and exporter of chilli in the international dry pepper market [10]. Chilli (*Capsicum annuum* L.) belongs to the Solanaceae family and is one of the most widespread spice crops with high commercial value. Pepper's history dates back to 7000 BC, when it was used in Mexico [11]. There are roughly 27 different types of chillies, but

the most often cultivated Capsicums are *Capsicum frutescense*, *Capsicum chinense*, *Capsicum pubescense*, and *Capsicum baccatum*. The main pepper-growing countries are North America, Africa, Latin America, Europe and Asia (FAOSTAT, 2013, FAOSTAT, 2019). Chilli is very nutritious, the fruit is rich in vitamins A and C, calcium, phosphorus, and iron, and contain capsaicin, which helps digestion [12], when green, it contains more vitamin C than citrus fruits, and red peppers contain more vitamin A than citrus fruits [13].

Streptomyces sp. is considered particularly an important work in the development of PGP activity to improve chilli growth. In the present study, *S. diastaticus* is used to produce auxin (IAA) in ISP-2 media. It is a promising alternative strategy to improve the growth and development of chilli by eliminating the use of artificial chemicals for the growth of chilli plant.

MATERIALS AND METHOD

Screening of *S. diastaticus* for IAA production

All the 18 isolates were tested for IAA production. IAA production for each isolate of *Streptomyces* was quantified using a colorimetric estimation [8]. Before IAA measurement, all *Streptomyces* isolates were

grown in 150 ml ISP2 liquid medium supplemented with 0.2% L-Tryptophan separately and incubated in an agitated incubator at 120 rpm at room temperature ($27\pm 2^\circ\text{C}$) for 5 days. About 1 ml of culture supernatant is mixed with 2 ml of Salkowski reagent [14] and then incubated in the dark for about 30 min. IAA production was observed by changing color from pink to red. IAA concentrations of all the isolates were calculated based on the standard curve. Each sample was tested in triplicates.

Plant Growth Promoting properties of *S. diastaticus* on Chilli

Phosphate solubilizing property of *S. diastaticus*

Phosphate solubilizing property was tested using Pikovskaya medium. Colony of *S. diastaticus* was spot inoculated onto the surface medium and incubated at $27\pm 2^\circ\text{C}$ for seven to ten days. A clear zone forming around the colony was evidence that phosphate was being dissolved. The following formula was used to determine the phosphate solubilization index [5].

$$\text{Phosphate Solubilization Index} = \frac{(\text{diameter of the colony} + \text{diameter of clear zone})}{\text{Diameter of colony}}$$

Qualitative test of *S. diastaticus* for ammonia generation

Freshly grown *S. diastaticus* was inoculated into 2 ml of peptone water and incubated at

$28\pm 2^\circ\text{C}$ for 7-12 days with shaking at 120rpm. After incubation 1 ml of Nessler's reagent was added in each culture tube. Development of yellow color indicates positive result for ammonia production [1].

Preparation of IAA standard curve

By comparing the isolates with the standard curve of known IAA concentrations and using Salkowski's reagent, the isolates were screened for quantitative production of IAA. Straight-line curve indicated direct relations between IAA concentrations. R2 value of the graph was found to be 0.979 that showed the validity of the graph [15].

Preparation of *S. diastaticus* Inoculum for Chilli Seed Treatment

Isolate was cultured in 250 ml conical flask containing ISP-2 broth and incubated in an orbital shaker at 120 rpm for 7 days at $28\pm 2^\circ\text{C}$ [16]. After incubation the culture was centrifuged at 1500 rpm for 2 minutes at 4°C . The Chilli seeds were surface sterilized with 2% H_2O_2 for 1 minute and washed for 4-5 times with sterile distilled water. Dry chilli seeds were immersed in the culture suspension for one hour. The treated seeds were spread on Petri plate and air dried overnight in laminar air flow [17].

Effect of *S. diastaticus* Seed Treatment on Germination and Vigour Index on Chilli.

Ragdoll method was applied for evaluating the PGP activity of rhizosphere *S.diastaticus* as described [7].

For the chilli treatment 7 days old culture filtrate of the isolate was used. Chilli seeds were surface sterilized by using 75% ethanol for 10 seconds and 2% of H₂O₂. Seeds were subjected for repeated washing (5-6 times) with sterilized distilled water. Further, the seeds were soaked for 60 minutes in sterile distilled water. The submerged seeds were selected and dipped in the filtered culture suspension of the isolate having CFU 2×10⁶ for 30 minutes. The soaked, germinated seeds were grown on a moist paper towel, folded, and then incubated for five days at 27±2° C. The seeds were treated sterile distilled water as a control. After the incubation period, three parameters were observed, including shoot length, root length, and number of lateral roots. The assay was conducted in triplicates, and each replication consists of ten seeds [18].

The germination percentage was recorded every day for 7 days and calculated by using the following formula.

$$\text{Germination rate (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

$$\text{Vigour index} = \% \text{ germination} \times \text{total plant length (root length + shoot length)}$$

RESULTS

Screening of *S.diastaticus* for IAA production

All the 18 isolates were able to produce IAA. Their concentration ranging from 0.45 to 8.15 µg/ml (Table 1). Among 18 isolated screened, the isolate SDSRO-2 produced the maximum IAA(8.15 µg/ml) and was chosen for the seedling vigour test because it was able to stimulate the growth of all three parameters such as lateral roots, root length, and shoot length in comparison to the control (Table 4 & 5).

Plant Growth Promoting properties of *S. diastaticus*

The efficacy of the potent *S. diastaticus* isolate in enhancing plant development was then evaluated. Isolate was able to be good promotive for all the three growth parameters of chilli seedlings over the control, which is treated with sterile distilled water (Figure 5).

Preparation of IAA Standard curve

IAA is prepared in concentrations ranging from 10 micrograms per millilitre to 100 micrograms per millilitre. 2 ml of Salkowski's reagent is added to the standard working solution, which is 1 ml. Readings are taken at 530 nm using a spectrophotometer after 25 minutes. IAA concentration in micrograms/ml against optical density at 530 nm is plotted to make a standard graph (Figure 3).

Table 1: The concentration of IAA produced by the 18 *Streptomyces* isolates in a 5days old ISP2 medium containing 0.2 ml of 0.2% of L-Tryptophan

No.	Isolate code	IAA concentration* (µg/ml)
1	SDSRO-1	3.8
2	SDSRO-2	8.15
3	SDSRO-3	6.6
4	SDSRO-4	2.7
5	SDSRO-5	3.3
6	SDSRO-6	1.8
7	SDSRO-7	5.35
8	SDSRO-8	1.85
9	SDSRO-90	4.05
10	SDSRO-10	1.4
11	SDSRO-11	0.45
12	SDSRO-12	1.2
13	SDSRO-13	4.6
14	SDSRO-14	6.3
15	SDSRO-15	2
16	SDSRO-16	5.75
17	SDSRO-17	1
18	SDSRO-18	1.3

Note: * The data were calculated from duplo measurement.

Among 18 isolates which were able to produce IAA. The isolate SDSRO-2 is found to be very high producer (8.15µg/ml) than the other rest whereas the least producer of IAA was SDSRO-11 (0.4µg/ml).

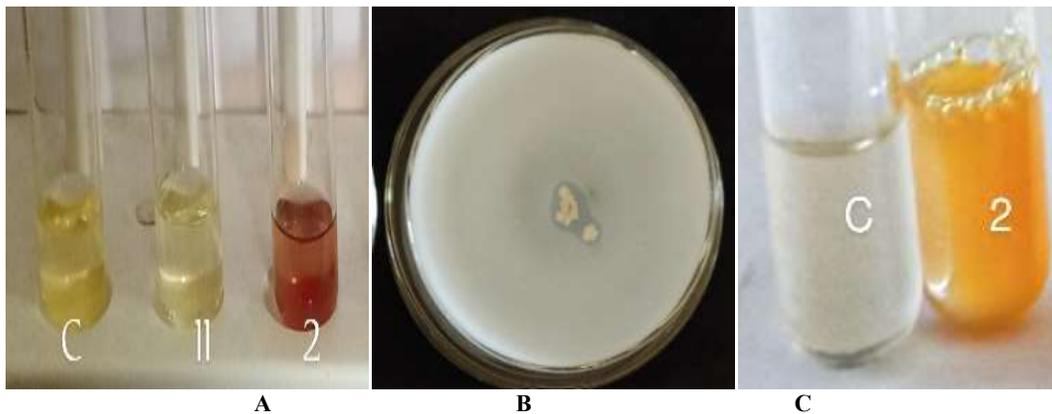


Figure 1: (A) Auxin Production (C-Control, 11- negative & 2-Positive *Streptomyces* isolates), (B) Phosphate solubilizing property of the isolate, (C) C-control, 2-Ammonia production by *S.diastaticus*

Table 2: In vitro screening results for IAA, Phosphate solubilization and Ammonia production

S. No.	IAA production	Phosphate solubilization	Ammonia production
Control	ND	-	ND
<i>S.diastaticus</i>	+	+	+

ND- No color development, '+' -positive, '-' -negative

Table 3: Phosphate solubilizing property of *S.diastaticus*

Isl. No.	Colony diameter(mm) (Average ± std dev)	Zone diameter(mm) (Average±std dev)	Phosphate solubilizing Index (average ±std dev)
SDSRO-2	0.65±0.070	0.39±0.106	2.515±0.070
SDSRO-6	0.42±0.062	0.13±0.012	1.309±0.007
SDSRO-15	0.3±0.12	0.10±0.10	1.333±0.212
SDSRO-12	0.16±0.03	0.01±0.012	1.062±0.014

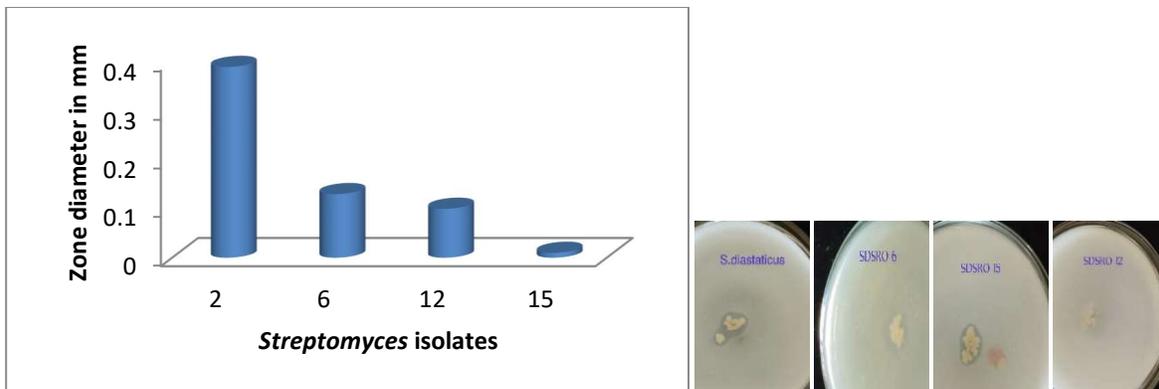


Figure 2: Zone diameter of Phosphate solubilization by the *Streptomyces* isolates

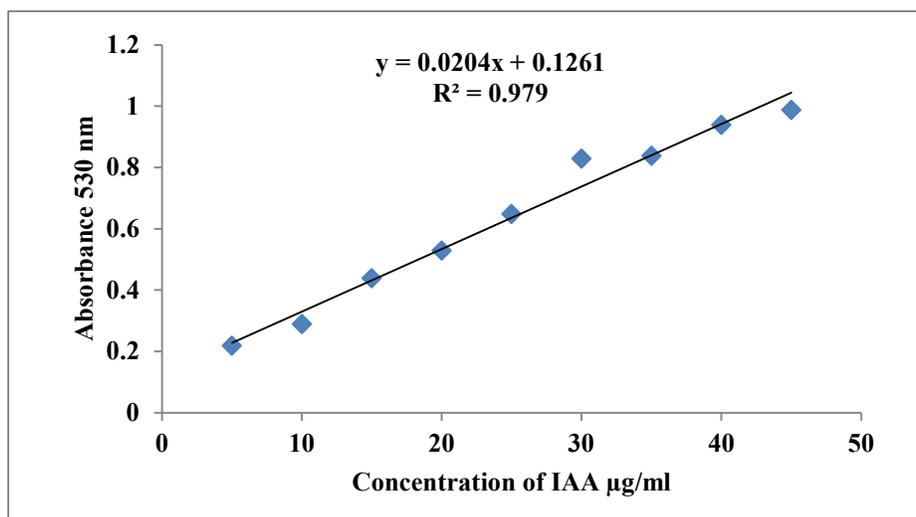


Figure 3: IAA standard curve

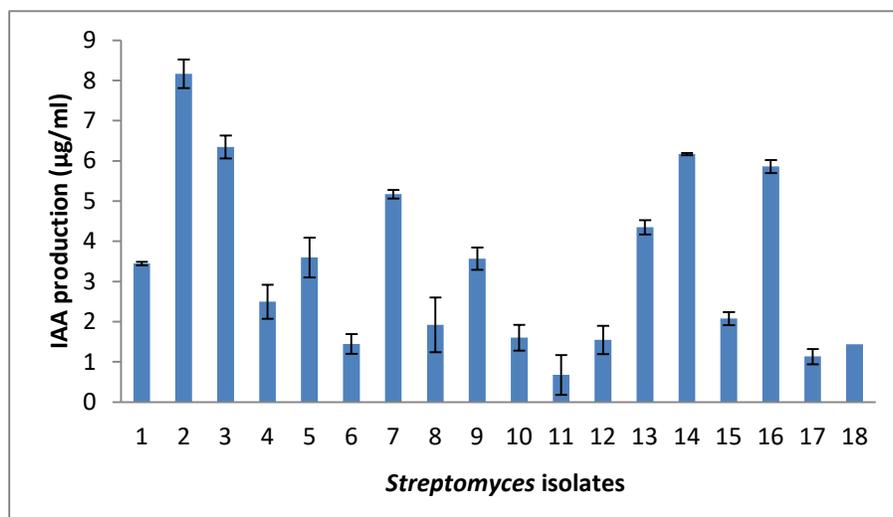


Figure 4: The concentration of IAA produced by the 18 *Streptomyces* isolates in a 5days old ISP2 medium containing 0.2 ml of 0.2% of L-Tryptophan (error bars± standard deviation)
 Note: * The data were calculated from duplo measurement

Table 4: Percentage of seed germination and Vigour index of Chilli

Treatment	% of seed germination (Average±std deviation)	Total seedling length (cm) (Average±std deviation)	Vigour index
Control	66.6±5.77	3.2±1.17	213.12 ±3.47
SDSRO-2	86.6±5.77	5.2±2.01	450.32 ±3.89

Table 5: Plant growth promoting activities in *In vitro* assay of *S.diastaticus*

Treatment	Root length (cm)* (Average±std deviation)	Shoot length (cm)* (Average±std deviation)	Number of lateral roots* (Average±std deviation)
Distilled water	2.4±0.15	4.1±0.15	2.3±0.57
<i>S. diastaticus</i>	3.8±0.16	6.7±0.36	6±1

* The data was calculated as the average of triplicates, each replication consists of 10 chilli seeds.

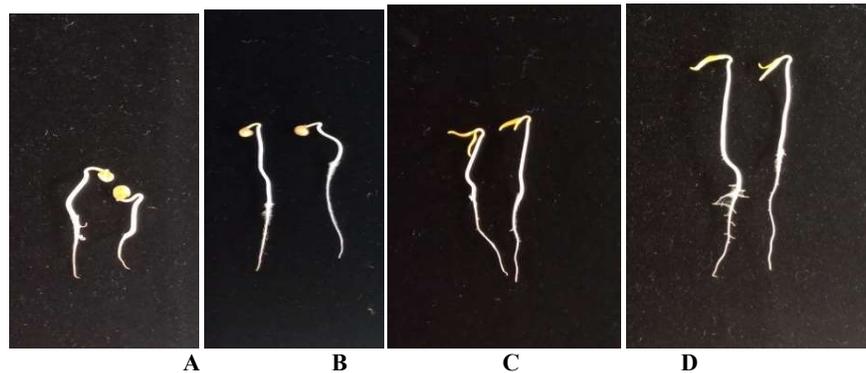


Figure 5: Chilli growth response after 5 and 7 days treated with: (A) control 5th day, (B) SDSRO-2 5th day, (C) control 7th day, (D) SDSRO-2 on 7th day

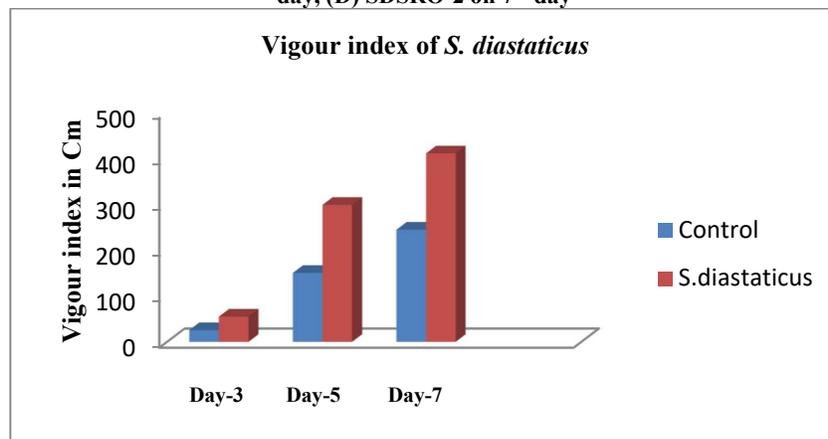


Figure 6: Vigour index of *S. diastaticus* on third, fifth and seventh days chilli seedlings *In vitro*

DISCUSSION

Actinomycetes are Gram-positive eubacteria with high G+C content of 78%. Majority of these can be isolated from the soil. In agriculture, actinomycetes are employed for plant growth promoting

activity. The present study aimed to screened for Plant Growth Promoting property of *Streptomyces diastaticus* which is isolated from the soil [25].

Four *Streptomyces* sp. from the selected isolates were capable of solubilizing phosphate by forming highest zone diameter (**Figure 1**) surrounding the colonies after seven days of incubation. The phosphate solubilization index of *Streptomyces diastaticus* (2.515 mm) was the highest (**Table 3**), followed by SDSRO-15 (1.33 mm) > SDSRO-6 (1.30 mm) > SDSRO-12 (1.06 mm) [1]. *S.diastaticus* also able to produce ammonia by developing yellow color (**Figure 1**) in peptone broth [26] suggested that bacteria can produce ammonia and supply nitrogen to the host plant. The ammonia produced by actinomycetes is beneficial for the root and shoot elongation, consequently increasing plant biomass. Moreover, it is very useful for the over production of ammonia which can serve as a triggering factor for the virulence of opportunistic plant pathogens [27].

Colorimetric estimation was employed for the IAA production [1]. Standard curve (**Figure 3**) was plotted of wavelength 530 nm [5]. The isolate was able to produce higher concentration of IAA that is 18.5 µg/ml. It is feasible that high concentration of L-Tryptophan will promote IAA production in the isolate which have isolated from the maize rhizosphere soil [19]. The effect of the

Streptomyces sp. on the growth of chilli seedlings was examined in the current study using the "ragdoll" method [7] with *S.diastaticus* showing the greatest increase in shoot and root lengths. The isolate was treated for the efficiency of PGP activity compared with the control. The experiment was conducted for 7 days and the chilli seedling growth response can be observed [4] (**Figure 5**).

The seedling vigour index was tested for the efficacy of the isolate on germination (**Figure 6**) the isolate showed 86.6% seed germination rate over control which is 66.6% and was carried out with 10 chilli seeds in triplicates (**Table 4**) [9]. The seeds were treated with seven days old cell-free culture suspension and control with distilled water for 7 days [20]. The data of root and shoot lengths (Centimeters) were measured from day third (**Figure 5**). Chilli seedlings showed highest growth response treated with the *S. diastaticus* when compared to the control [21]. The highest growth response of chilli was due to the availability of L-tryptophan in the medium that provides the precursor for IAA synthesis [22]. In this study, SDSRO-2 and SDSRO-1 produced the highest and lowest IAA concentrations, respectively. Such differences are likely influenced by the genetic [25] and metabolic

background of each isolate in converting L-tryptophan to IAA [26]. It is hypothesized that the rhizosphere-isolated strain of *Streptomyces* sp. can synthesize IAA [23]. IAA controls some vital development of the plant including cell division, cell expansion, root development, and apical dominance [23] [24]. The agronomic characteristics of the *Streptomyces* strain employed in this study increased significantly compared to the uninoculated control, comprising shoot length, root length, number of lateral roots and plant height [27].

CONCLUSION

Present study revealed there are effective P-solubilizing, Ammonia production, and IAA-producing Actinomycetes are ubiquitous in the natural population. These qualities are regarded as essential PGP characteristics and their effectiveness is positively increasing the total yield and development of the evaluated chilli plants. The study's findings demonstrate that rhizosphere soil is a rich source of IAA-producing *Streptomyces* sp. So, it can be stated that the presence of such growth-promoting actinomycetes is responsible for the beneficial effects on plant growth in agricultural fields, and they can be used as biofertilizers instead of industrial chemicals. In the present investigation, *S. diastaticus* IAA-producing isolate

enhanced plant growth in terms of increasing root and shoot lengths, as compared to uninoculated control chilli seedlings. According to the potential plant growth promoting property of *S. diastaticus* it needs to be further developed as a biofertilizer agent for sustainable agriculture, especially on chilli crops. The outcomes of this study may be taken into account when developing a strategy for using *Streptomyces diastaticus* as a PGP agent.

ACKNOWLEDGEMENT

Authors thank for the Principal Sahyadri Science College Shivamogga for providing good laboratory facility for the research work.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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