Recent Trends in PGPR Research for Sustainable Crop Productivity

Riyaz Z. Sayyed
Munagala S. Reddy
Ahmad I. Al-Turki

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UTILIZATION OF ACTINOMYCETES HAVING BROAD-SPECTRUM OF PLANT GROWTH-PROMOTING AND BIOCONTROL TRAITS IN CHICKPEA, SORGHUM AND RICE

A. Sathya*, V. Srinivas and S. Gopalakrishnan**

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502324, Telangana, India.

**E-mail: A.satya@cgiar.org, s.gopalakrishnan@cgiar.org*

ABSTRACT

Plant pathogens such as Sclerotium rolfsii (causes collar rot), Fusarium oxysporum (causes wilt) and Macrophomina phaseolina (causes charcoal rot/dry root rot) have a broad host range, affecting several agriculturally important crops including chickpea, pigeon pea, groundnut and sorghum, which are grown under rainfed conditions, leading to significant yield losses. Due to the broad host range of these fungal pathogens, it has become very difficult for the farmers to grow these crops profitably. Hence, there is a need to have broad-spectrum plant growth-promoting (PGP) and biocontrol organisms for use in different cropping systems for the control of multiple diseases in a single crop and there by the crop productivity can be enhanced in the dry-land agriculture. The main objective of the present study was to identify and evaluate broad spectrum PGP and biocontrol agents and their metabolites with multiple actions against different pathogens so that one biological treatment controls more than one problem apart from promotion of plant growth in chickpea, sorghum and rice.

INTRODUCTION

Agricultural sector is facing burden from many ways via lower soil nutrients, attack of pathogen, pest and weeds and fluctuating climatic conditions which leads to economic consequences. Among them, pathogenic microbes are one of the major threat for food production and also ecosystem stability; because a single crop is affected by multiple pathogens, and vice versa i.e., many phyto-pathogens have broad host range and hence affect multiple crops. Ex Chickpea is affected by multiple pathogens including (i) bacteria: *Xanthomonas campestris* - bacterial blight, (ii) fungi: *Ascochytar abiei* - *Ascochyta* blight; *Botrytis cinerea* - *Botrytis* gray mold;

Alternaria alternata – Alternaria blight; *Colletotrichum dematium* - *Colletotrichum* blight, *Sclerotinia sclerotiorum* - *Sclerotinia* stem rot; *Fusarium oxysporum* - *Fusarium* wilt; *F. solani* -black root rot; *Sclerotium rolfsii* - collar rot; *Rhizoctonia solani* - wet root rot, and (iii) virus: Stunt - leaf roll virus; Narrow leaf - yellow mosaic virus; Necrosis - necrotic yellows virus (Nene et al. 2012). Pathogens like *Aschochyta* and *Botrytis* have the host range of around 50 (Skoglund et al. 2011) and 200 (Williamson et al. 2007) plant species, respectively. To overcome this multi-pathogen attacks, farmers are in a situation to increase the use of chemical inputs which further leads to pathogen resistance against the agents and other non-target environmental impacts (Compant et al. 2005). Besides this, increasing human population requires higher productivity of food crops since plants are the basis for every food chain.

In view of the above status, strategies involving plant productivity in an environmentally sustainable manner is necessary in which biological options are of great importance. The antagonistic or plant growth-promoting (PGP) rhizobacteria and their bioactive antimicrobial compounds are considered as environmentally safe and easily biodegradable (Morrissey et al. 2004). PGP microbes are those colonizing the root surfaces and the closely adhering soil interface i.e., the rhizosphere or exist in internal tissues as endophytes and helps in promoting plant growth by various mechanisms. This includes direct (nitrogen fixation, phosphate solubilization, iron chelation and phytohormone production) or indirect (suppression of pathogens, induction of resistance in host plants against pathogens and abiotic stresses) mechanisms. Some of the representatives of PGP microbes are *Bacillus, Pseudomonas, Erwinia, Caulobacter, Serratia, Arthrobacter, Micrococcus, Flavo-bacterium, Rhizobium, Bradyrhizobium, Sinorhizobium,* and *Streptomyces* (Vessey 2003; Ma et al. 2011).

The present study was designed to evaluate PGP microbes isolated form vermicompost for their role in growth promotion on chickpea, rice and sorghum and their antagonistic potential on plant pathogens.

MATERIALS AND METHODS

Microorganisms

Five strains of *Streptomyces* sp., CAI-24 (NCBI accession: JN400112), CAI-121 (NCBI accession: JN400113), CAI-127 (NCBI accession: JN400114), KAI-32(NCBI accession: JN400115), and KAI-90 (NCBI accession: JN400116) form Microbial germ plasm collection at ICRISAT were selected for the study.

In vitro plant growth-promoting traits

The isolates were tested for their PGP traits by the estimation of indole acetic acid (IAA) (Patten & Glick 1996), siderophore (Schwyn & Neilands 1987), phosphate utilization (Nautiyal 1999) and lytic enzymes such as cellulase (Hendricks et al. 1995), protease (Bhattacharya et al. 2009), chitinase (Hirano & Nagao 1988), β-1,3-glucanase production (Singh et al. 1999) and other molecules such as hydrocyanic acid (HCN) (Lorck, 1948). The isolates were also tested for their antagonistic activity

against *Fusarium oxysporum* f. sp. *ciceri* (FOC) and *Macrophomia phaseolina* (Tassi) Goid., (MP) for checking their broad-spectrum potential.

Evaluation of Streptomyces for PGP properties

Chickpea

The five actinomycetes isolates CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were evaluated for their PGP properties on chickpea variety ICCV2 under field conditions at 2012-2013 at ICRISAT, Telangana, India. The seeds were treated with *Streptomyces* culture (10^8 CFU ml^{-1}) for 45-50 min and sown immediately. Plants were inoculated with respective *Streptomyces* strains (1000 ml; 10^8 CFU ml^{-1}) once every 15 days on the soil close to the plant until the flowering stage. All the agronomic practices were done as and when required. Growth parameters were measured at regular intervals. The details of this trial can be found at Gopalakrishnan et al. (2015).

Rice

Effect of *Streptomyces* sp., CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 on growth performance of rice was performed in the 2011 Kharif (rainy) season at ICRISAT under system of rice intensification (SRI) method (Uphoff 2003). The 11-day-old single seedlings were uprooted from the nursery; their roots were dipped in respective *Streptomyces* culture (containing 10^8 cfu ml^{-1}) for 60 min and transplanted in to the field. Plants were inoculated with respective *Streptomyces* strains (1500 ml; 10^8 cfu ml^{-1}) once in every two weeks until the flowering stage. All the agronomic practices were done as and when required. Growth parameters were measured at regular intervals. The details of this trial can be found at Gopalakrishnan et al. (2013).

Sorghum

Growth promoting ability of the five *Streptomyces* isolates on sorghum variety ICSV112 was evaluated under greenhouse conditions. The seeds were treated with *Streptomyces* isolates (10^8 CFU ml^{-1}) for 45-50 min and sown immediately. Booster doses of *Streptomyces* strains (5 ml per seedling, 10^8cfu ml^{-1}) were applied at 15days interval by the soil drench method. Growth parameters were evaluated at regular intervals. The details of this experiment can be found at Gopalakrishnan et al. (2013).

Characterization for colonization and gene expression

Colonization ability of *Streptomyces* isolates on chickpea roots were done as per the protocols of Bozzola and Russell (1998) and the roots were examined under scanning electron microscope (JOEL-JSM5600). Gene expression profile of these isolates were tested for IAA, β-1,3-glucanase and siderophore genes and their transcription levels were measured as per the protocols of Gopalakrishnan et al. (2014).

Evaluation of Streptomyces for biocontrol properties

Fusarium wilt of chickpea

The five *Streptomyces* sp., CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were evaluated for biocontrol potential against FOC under greenhouse conditions on chickpea variety JG-62. FOC inoculum was added at the concentration of 20% of pot weight and subjected to the following treatment methods: M1 - inoculation of thepotting mixture with respective actinomycete culture along withFOC two weeks before sowing; M2 - inoculation of the seeds by soaking in the respective actinomycete culture for 1 h; M3 - inoculation of the sprouted seeds by soaking in the respective actinomycete culture for 1 h; M4 - inoculation of the potting mixture with actinomycete culture at the time of sowing (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seed and covered with soil) and M5 - inoculation of the seedlings after emergence with actinomycete culture (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seedlings after emergence). Similarly, isolates were also tested in *Fusarium*-infested field at ICRISAT during 2009-10 cropping seasons with all the inoculation methods except M1. Disease symptoms and growth parameters were evaluated at regular intervals. The details of this trial can be found at Gopalakrishnan et al. (2011).

Charcoal rot of sorghum

The five *Streptomyces* sp., CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were evaluated for biocontrol potential against MP under greenhouse conditions on sorghum. The seeds were treated with *Streptomyces* isolates (10^8 cfu ml^{-1}) for 45-50 min and sown immediately. Booster doses of *Streptomyces* strains (5 mlper seedling, 10^8cfu ml^{-1}) were applied at 15days interval by the soil drench method. The disease was induced by inserting toothpick infested with MP inoculum in the second internode of stalk at 10-15 days after flowering (Das et al. 2007). After 25-30 days of disease induction, the symptoms were measured at 1-5 scale.

RESULTS AND DISCUSSION

Plant growth promoting traits - in vitro

All of the *Streptomyces* sp., CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were invariably found to produce siderophore, HCN, IAA, β-1,3-glucanse and lipase *in vitro* (Fig. 1). Other PGP properties such as cellulase (only in KAI-32 and KAI-90), protease (only in CAI-24 and CAI-127) and chitinase (only in CAI-24, KAI-32 and KAI-90) were also observed. Inhibitory activity of these isolates was documented against FOC and MP. Further details of this can be found at Gopalakrishnan et al. (2011 and 2013).

HCN, hydrocyanic acid; IAA, indole acetic acid; Values on primary (left) axis are rating scales for in vitro PGP traits of (i) siderophore, chitinase, cellulase, lipase and protease production as: 0, no halo zone; 1, halo zone of <1 mm; 2, halo zone of 1–3 mm; 3, halo zone of 4–6 mm and 4, halo zone of7 mm and above; (ii) chitinase production as: 0, no halo zone; 1, halo zone of 1–5 mm; 2, halo zone of 6–10 mm, 3,

halo zone of 11–15 mm; 4, halo zone of 16–20 mm and 5, halo zone of 21 mm and above; (iii) HCN production as: 0, no color change; 1, light reddish brown; 2, medium reddish brown and 3, dark reddish brown; (iv) β-1,3-glucanase (U) – one unit is an amount of enzyme that liberated 1 µmol of glucose hour^{-1} at defined conditions. Values on secondary (right) axis indicate IAA production.

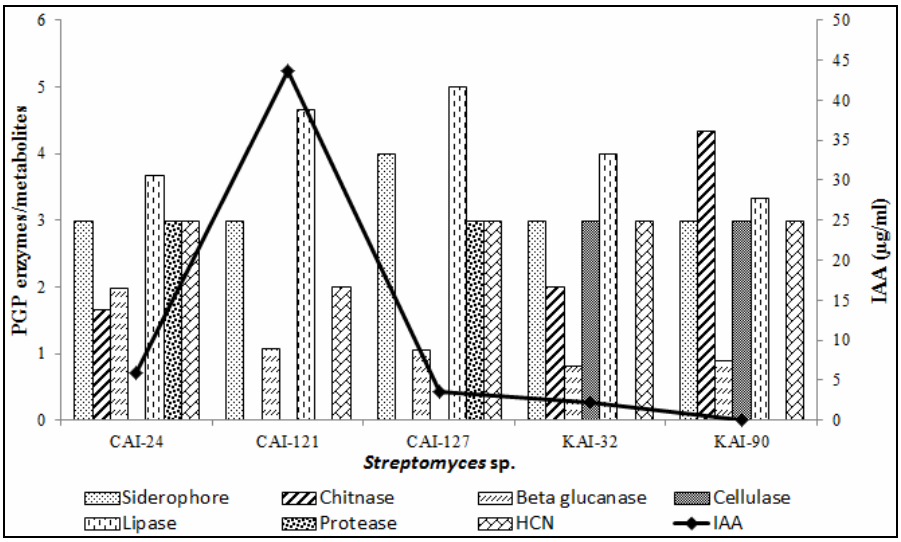

Fig. 1 Plant growth promoting and biocontrol traits of *Streptomyces* sp.

Plant growth promoting properties of Streptomycessp.- Greenhouse and field conditions

Chickpea

All the *Streptomyces* strains were found to enhance agronomic performance of chickpea (Table 1). After 30 days of sowing, the nodule number, nodule weight and root weight was increased up to 70, 82 and 6%, respectively over the control plots. At 60 days after sowing, pod number and pod weight was increased up to 51 and 85%, respectively than control. At harvest, 39 and 12% increase was observed for stover yield and grain yield over the control treatments.

Table 1. Effect of PGP *Streptomyces* sp. on agronomic performance of chickpea

Isolates	At 30 days of sowing			At 60 days after sowing		At harvest	
	Nodule number (plant $^{-1}$)	Nodule weight (mg plant^{-1})	Root weight (mg plant $^{-1}$)	Pod number (plant $^{-1}$)	Pod weight (g plant $^{-1}$)	Stover yield (t ha^{-1})	Grain yield (t ha^{-1})
CAI-24	14	58	175	65	3.05	1.24	1.83
CAI-121	25	53	176	63	4.16	1.67	1.87
CAI-127	17	31	176	59	2.89	1.53	1.8

KAI-32	17	48	184	68	4.44	1.42	1.8
KAI-90	31	75	196	68	4.25	1.82	2.07
Control	12	29	171	43	2.04	1.11	1.68
Mean	19	48	180	61	3.47	1.46	1.84
SE	1.5***	4.1***	3.5***	2***	0.206***	0.03***	0.013***
LSD	4.7	12.8	10.9	6.3	0.64	0.095	0.042
CV%	13	14	3	6	10	4	1

LSD – Least Significant Difference; CV – Co-efficient of Variation;
*** - Significant at $p < 0.001$

The highest increase of agronomic traits was observed in *Streptomyces* sp., KAI-90 treated plot. This might be due to their plant growth promoting traits which were proved *in vitro*; on the other hand, the isolates also increased soil parameters like total N (5%), available P (37%) and organic carbon (9%) than control treatments (Table 2). Microbial action on these influences was proved by higher microbial biomass carbon (55%) and dehydrogenase activity (17%) in rhizospheric soil of *Streptomyces* treated chickpea cultivation fields than control treatments (Table 2). Microbial action on agronomic performance was further revealed by up-regulation on gene expression of PGP genes including IAA (10 fold) and siderophore (12.6 fold) and also biocontrol genes, β-1,3-glucanse (2.4 fold) (Fig. 2). They also documented for colonization capacity on chickpea roots (Fig. 3), which helps in competing against pathogens and hence provide protection against fungal pathogens (Gopalakrishnan et al. 2015).

Table 2. Effect of PGP *Streptomyces* sp. on rhizospheric soil health at harvest of chickpea

Isolates	**MBC (µg g^{-1} soil)**	**DA (µg TPF g^{-1} soil 24 h^{-1})**	**Total N (ppm)**	**Available P (ppm)**	**OC (%)**
CAI-24	1041	71.1	668	10.3	0.5
CAI-121	1157	58.9	667	11.1	0.54
CAI-127	1070	59.1	644	19.3	0.52
KAI-32	1430	58.8	642	17.8	0.5
KAI-90	1134	62.7	701	10.9	0.49
Control	752	53.1	632	10.1	0.47
Mean	1097	60.6	659	13.2	0.5
SE	63.5**	0.66**	7.6**	0.24***	0.005**
LSD	231	2.4	27.7	0.88	0.019
CV%	8	3	2	3	2

MBC - Microbial biomass carbon; DA - Dehydrogenase activity; OC - Organic carbon;
LSD – Least Significant Difference; CV – Co-efficient of Variation; ** - Significant at $p < 0.01$; *** - Significant at $p < 0.001$

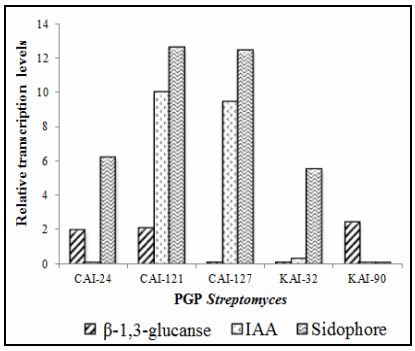

Fig. 2 Expression profile of PGP genes of *Streptomyces* sp.

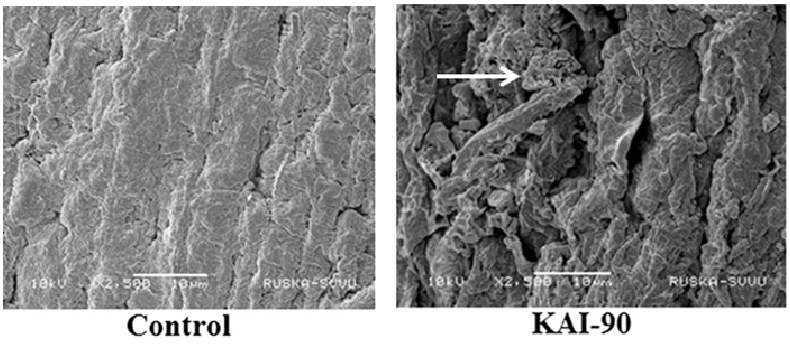

Fig. 3. Scanning electron microscopy images of the control & and *Streptomyces* sp., KAI-90 treated chickpea roots. Arrow indicates colonization of KAI-90 on chickpea roots.

Rice

All the *Streptomyces* sp., were found to enhance rice growth tested under SRI cultivation method (Table 3). During the harvest period, an increase of up to 28%, 18%, 25% and 10% was observed on tiller numbers, panicle numbers, stover yield and grain yield over the control treatment. Root development parameters including root length (up to 15%), root volume (up to 35%) and root dry weight (up to 55%) were influenced by the PGP strains.

Table 3. Effect of PGP *Streptomyces* sp. on agronomic performance of rice, at harvest

Isolates	Tiller numbers (m^{-2})	Panicle number	Grain yield (g m^{-2})	Stover yield (g m^{-2})	Root length (m m^{-2})	Root volume (cm^{-3} m^{-2})	Root dry weight (g m^{-2})
CAI-24	586	41.7	587	584	2087	396	30.5
CAI-121	506	38.3	583	637	3263	513	36
CAI-127	501	43.1	619	754	3470	692	49.5
KAI-32	532	40.5	640	754	3652	627	54.3
KAI-90	589	39.5	587	693	3562	581	44.8

Control	459	36.4	582	601	3182	507	35.1
Mean	529	39.8	600	671	726	88	5.9
SE	15.6***	0.29***	9.7**	27.8**	81.6***	16.8***	1.89***
LSD	47.1	1.06	30.5	87.8			
CV%	7	2	3	7	7	7	10

LSD – Least Significant Difference; CV – Co-efficient of Variation; ** - Significant at $p < 0.01$; ** - Significant at $p < 0.001$

In addition, soil properties were influenced by PGP microbes which were shown up to 122%, 53% and 13% increase of total N, available P and organic carbon over the control treatment (Table 4). Role of microbes in increasing these soil parameters is evidenced by higher concentration of microbial biomass up to 41%, microbial biomass nitrogen up to 52% and dehydrogenase activity up to 75% over the un-inoculated plots. The highest increase of agronomic traits was observed in *Streptomyces* sp., KAI-32 treated plot (Gopalakrishnan et al. 2013).

Sorghum

The five *Streptomyces* sp., strains significantly enhanced PGP parameters of sorghum by increasing plant height (up to 51%), stem weight (up to 39%) and root length (up to 18%), root volume (up to 9%) and root dry weight (up to 25%) over the control treatment (Table 5; Gopalakrishnan et al. 2013).

Table 4. Effect of PGP *Streptomyces* sp. on rhizospheric soil health, at harvest of rice

Isolates	**MBC ($\mu g\ g^{-1}$ soil)**	**MBN ($\mu g\ g^{-1}$ soil)**	**DA (μg TPF g^{-1} soil24 h^{-1})**	**Total N (gKg^{-1}soil)**	**Available P ($mg\ g^{-1}$ soil)**	**OC (%)**
CAI-24	1715	60	94	2.456	0.133	1.49
CAI-121	3293	65	113	1.992	0.117	1.47
CAI-127	2875	88	194	2.644	0.115	1.52
KAI-32	4020	65	135	2.142	0.122	1.66
KAI-90	2946	62	136	2.16	0.129	1.62
Control	2861	58	111	1.19	0.087	1.47
Mean	2952	66	131	2.231	0.117	1.53
SE	150.5***	3.7**	9.3***	0.063*	0.005*	0.032*
LSD	520.7	12	29.7	0.229	0.019	0.116
CV%	9	10	12	4	6	3

MBC - Microbial biomass carbon; MBN - Microbial biomass nitrogen; DA - Dehydrogenase activity; OC - Organic carbon; LSD – Least Significant Difference; CV – Co-efficient of Variation; * - Significant at $p < 0.05$; ** - Significant at $p < 0.01$; ** - Significant at $p < 0.001$

Table 5. Effect of PGP *Streptomyces* sp. on growth performance of sorghum under greenhouse conditions

Isolates	Plant height (cm)	Stem weight (g)	Root length (m plant^{-1})	Root volume (cm^3 plant^{-1})	Root dry weight (g plant^{-1})
CAI-24	114	5.42	90	10.2	0.92
CAI-121	122	5.55	80	9	0.84
CAI-127	100	5.09	88	9.3	0.87
KAI-32	124	6.39	82	10.5	0.89
KAI-90	130	7.03	78	10.5	1.01
Control	86	5.06	76	8.8	0.81
Mean	113	5.76	82	9.7	0.89
SE	4.9***	0.471*	3.4*	0.42**	0.036*
LSD	14.9	1.421	10	1.28	0.109
CV%	9	16	8	9	8

LSD – Least Significant Difference; CV – Co-efficient of Variation; * - Significant at $p < 0.05$; ** - Significant at $p < 0.01$; ** - Significant at $p < 0.001$

Biocontrol potential of PGP Streptomyces

Fusarium wilt of chickpea

All the 5 PGP *Streptomyces* sp., showed inhibitory activity against FOC under laboratory conditions. Under green house conditions, up to 76% reduction in disease incidence was observed at 29 days after sowing (DAS) with highest reduction of 76% in *Streptomyces* sp., CAI-24 which showed good antagonistic activity on M1, M2 and M5 methods of inoculation (up to 76%).

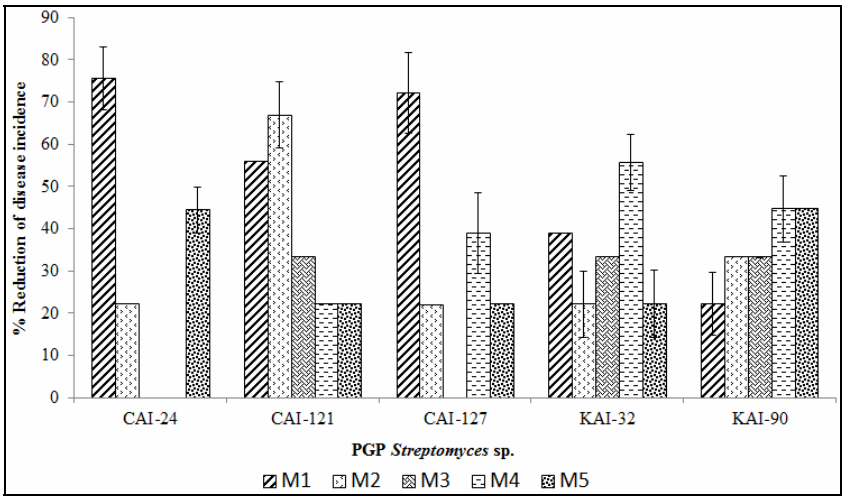

Fig. 4 Effect of PGP *Streptomyces* sp., on disease incidence of *Fusarium*wilt of chickpea under greenhouse conditions at 29 DAS

M1 - inoculation of the potting mixture with respective actinomycete culture along with FOC two weeks before sowing; M2 - inoculation of the seeds by soaking in the respective actinomycete culture for 1 h; M3 - inoculation of the sprouted seeds by soaking in the respective actinomycete culture for 1 h; M4 - inoculation of the potting mixture with actinomycete culture at the time of sowing (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seed and covered with soil) and M5 - inoculation of the seedlings after emergence with actinomycete culture (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seedlings after emergence).

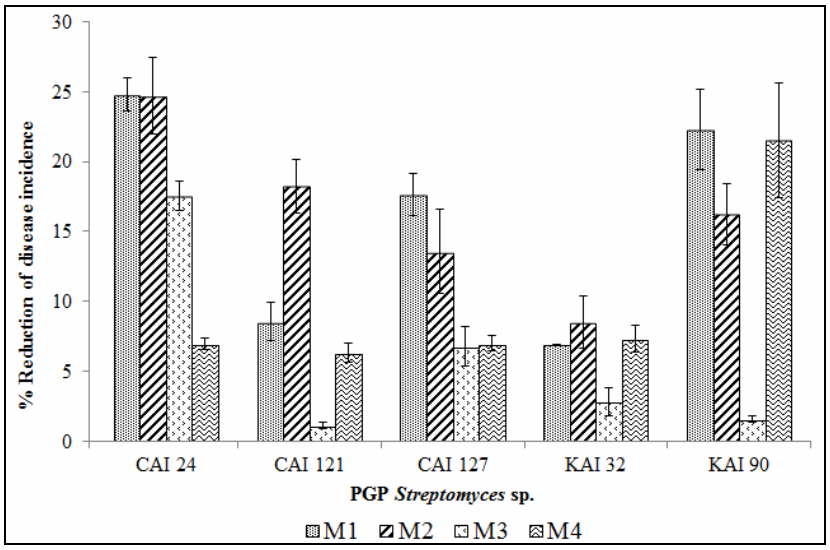

Fig. 5 Effect of PGP *Streptomyces* sp., on disease incidence of *Fusarium* wilt of chickpea under wilt sick plots at 24 DAS

M1 - inoculation of the seeds by soaking in the respective actinomycete culture for 1 h; M2 - inoculation of the sprouted seeds by soaking in the respective actinomycete culture for 1 h; M3 - inoculation of the potting mixture with actinomycete culture at the time of sowing (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seed and covered with soil) and M4 - inoculation of the seedlings after emergence with actinomycete culture (10 ml of actinomycete culture [10^8 CFU ml^{-1}] was applied on the seedlings after emergence).

Remaining isolates CAI-121, CAI-127, KAI-32 and KAI-90 reduced the disease incidence on all the 5 methods of actinomycete inoculation up to 67%, 72%, 56% and 45%, respectively, whereas in control plots all the plants got killed by 21 days (Fig. 4). The same isolates also reduced the disease incidence on chickpea under wilt sick plots up to 19% on 28 DAS with the maximum degree of protection by CAI-24 followed by KAI-90. In un-inoculated control plots 100% disease incidence was noticed earlier by 20 DAS.

Among the methods of inoculation, M1 i.e., seed inoculation was found to best (Fig. 5). This might be due the production of inhibitory metabolites such as HCN and hydrolytic enzymes such as chitinase, cellulase, lipase and β-1,3-glucanse which were observed by laboratory analysis and/or gene expression (Gopalakrishnan et al. 2011).

Charcoal rot of sorghum

All of the PGP *Streptomyces* sp., documented biocontrol traits against *M. phaseolina* on sorghum under greenhouse conditions with 20-81% reduction in charcoal rot extension. The highest reduction of 81% was noticed on KAI-90 treated sorghum plants (Fig. 6). These results are yet to be published and the field evaluation trials are in progress.

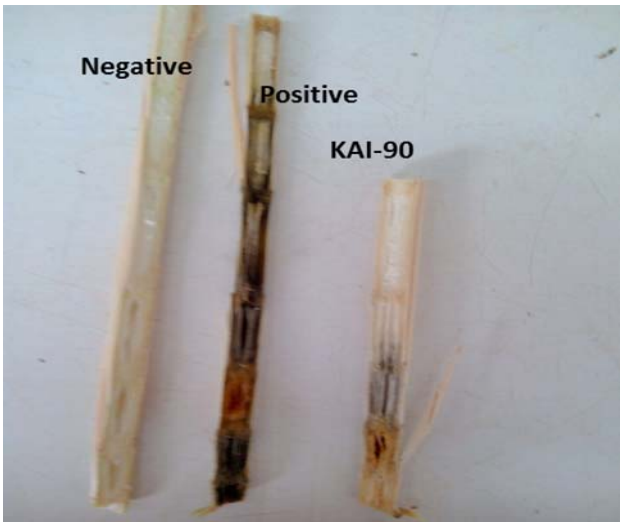

Fig. 6 Charcoal rot reduction of *Streptomyces* sp., KAI-90 treated sorghum

CONCLUSION

The five *Streptomyces* sp., evaluated in the present study documented plant growth promoting traits *in vitro.* They also enhanced the growth and yield in chickpea and rice under field conditions and sorghum under greenhouse conditions. The isolates also possess biocontrol potential against FOC and MP. This was evidenced by the reduced disease incidence on chickpea and sorghum under wilt sick plots and greenhouse conditions, respectively. Colonization ability on chickpea roots and up-regulation of PGP gene such as IAA, siderophore and β-1,3-glucanases are the other identified evidences for their role in both plant growth promotion and biocontrol potential. This study reveals the importance of PGP actinomycetes as broad spectrum growth promoting and biocontrolagents as they proved their effect on multiple crops and pathogens. So, research and development of potential broad spectrum PGP microbes will provide greater understanding of the multiple facets of disease suppression and offer significant strategies for disease management and crop productivity for sustainable agriculture in the near future.

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