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Two *Streptomyces* spp. as *Rhizoctonia bataticola* Antagonists and Seedling Growth Promoters for Soybean (*Glycin max* (L.) merill)

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A B S T R A C T

Streptomyces spp. ANSP4 and ANSCa22 isolated earlier from rhizospheric soil of Akola district of Maharashtra were studied for their biocontrol potential against *Rhizoctonia bataticola* isolates from the same region. Both *Streptomyces* strains ANSP4 and ANSCa22 revealed growth inhibition of *Rhizoctonia bataticola* isolates R1 and R2 by dual culture method. Efficacy of independent applications of these strains on seedlings vigor index (SVI) of R1 inoculated Soybean seeds were tested by paper towel method. R1 inoculation of healthy soybean seeds resulted in drastic reduction in SVI from 1515.88 to 666.75. Independent treatments of R1 inoculated seeds with *Streptomyces* sp. ANSP4 and *Streptomyces* sp. ANSCa22 resulted in increase in SVI up to 2014.88 and 1928.64 respectively. This increase in SVI was significantly greater as compared to the treatment with Carbendazim. Treatments of both these cultures had enhancing effects on growth characteristics and germination percentage of soybean seeds as compared to control .Increase was recorded in average root length and shoot length .Roots developed after biological treatments were healthier than the roots developed after chemical treatment and control.

Keywords

Antagonistic activity, Streptomyces spp., Rhizoctonia bataticola, plant growth promotion

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Introduction

Soybean is the most important oil seed and pulse crop in the world and is a major crop of few states of India. *Rhizoctonia bataticola* (pycnidial stage *Macrophomina phaseolina*) is a soil born pathogen that causes many diseases such as charcoal rot, root rot, seedling blight, collar rot and damping off. High yield losses were reported due to charcoal rot caused by this pathogen in soybean (Mengistu et. al., 2011). Root rot incidences in major soybean growing states in India were reported to be in the range of 3.29% to 40.33% (Belkar et. al., 2016). Seedling infection is common and also fatal to a large extent. Severity of the damage due to *Rhizoctonia* was reported to be more in young seedlings as compared to other stages

(Kumar et. al., 2019). Chemical fungicides have been commonly employed to mitigate the losses due to diseases caused by fungal phytopathogens (Brauer et.al.2019). Inspite of their benefits for plant disease control, intensive efforts are focused worldwide for limiting the use of chemical fungicides considering their toxicity to non target plants, animals and micro flora. Some of the synthetic chemical fungicides have been found to show carcinogenicity, mutagenicity and teratogenicity (Sharma 2015).

Exploring antagonistic microorganisms for control of phytopathogens is an environmentally sustainable strategy (Heydara and Pessarakli, 2010). Many bacterial genera including *Pseudomonas*, *Bacillus* and *Streptomyces* have been investigated by researchers for their application potential against common phytopathogens (Bouizgarne,2013) .

Streptomyces is a bacterial genus extensively exploited for their abilities to produce a variety of secondary metabolites that include antifungal and plant growth promoting compounds (Doumbou et. al., 2001). Several reports indicate the antagonistic properties of *Streptomyces* spp. against fungal phytopathogens (Ranveer et. al., 2005).

Rhizospheric competence of any introduced bacteria largely depends on physical, chemical and biological factors prevailing in the soil. The chances of selecting potentially field effective strains are greater if the bacterial strains are isolated from the same environment where their application is desired (Weller 1998).

Keeping in view all the above factors, some *Streptomyces* spp. were isolated previously from the non saline rhizospheric soil of some plants grown in Kanheri sarap village of Akola district, India (Khendkar and Deshpande, 2018) Present study was undertaken to evaluate the efficacy of *Streptomyces* sp. ANSP4 and *Streptomyces* sp. ANSCa22 treatments for controlling *Rhizoctonia bataticola* infection and seedling growth promotion of soybean.

Materials and Methods

Isolation and identification of *Rhizoctonia bataticola*

Infected Soybean plant samples were collected from diseased plants from Akola district. Samples were cut into small pieces. Surface sterilized sample pieces were kept on PDA and incubated for 7 days at 27°C. After incubation fungal colonies were identified on the basis of colony appearance and microscopy (Veena *et al.*, 2014).

Test of virulence of *R. bataticola* isolates

Inoculum of *R. bataticola* was mixed into sterilized soil at 4% by weight of soil (Lukade, 1992) and was filled in sterilized pots. Surface sterilized Soybean seeds were used for sowing. Soil without inoculum of pathogen was used as control.

Readings were noted on 15th day after sowing regarding germination % and number of survived and infected plants and then disease incidence was calculated (Srinivas, 2016).

Evaluation for antagonistic potential

Dual culture method

The *Streptomyces* strains were tested for their antifungal activity against *Rhizoctonia bataticola* isolates R1 and R2 using dual culture method by spot inoculating *Streptomyces* strains on starch casein agar plate spread inoculated with the pathogen. Zones of inhibition were recorded.

Preparation of inoculum and treatments

Streptomyces sp. ANSP4 was grown on Starch casein broth for about 7 days at 30°C. Biomass was collected and 20% biomass was mixed with talcum powder. Same procedure was followed for *Streptomyces* sp. ANSCa22.

Fresh inoculum of *R. bataticola* (R1) was prepared in potato dextrose broth. Surface sterilized seeds were socked in fresh biomass of *R. bataticola* and left for 30 minutes (T2).

Then the seeds were mixed with powder formulation and used for the study. R1 inoculated seeds were separately treated by ANSP4 (T1), ANSCa22 (T4) and Carbendazim (T3). Seeds without any treatment served as Control(C).

Paper towel method

Paper towel method (Chaithra, 2009) was performed to evaluate the effect of different treatments on seedling vigour index (SVI) of soybean seeds. Germination paper sheet was lightly moistened with sterilized distilled water and surfaces sterilized Soybean seeds (50) with and without treatments were kept on germination paper.

This paper was covered with another germination paper sheet and it was moistened with sterile distilled water. The paper set was rolled, loosely tide by thread and kept in beaker containing sterile water. Results were noted after 10 days and SVI was calculated as per following formula (Ingle, 1999). Seedling vigor index (SVI) = Mean of shoot length+ Mean of root length x germination percent

Results and Discussion

The strains of *Rhizoctonia bataticola* (R1- R4) were isolated from the infected soybean plants of Kanheri sarap of Akola district. Virulence of four isolates of *Rhizoctonia bataticola* (R1 to R4) when tested by sick soil method for soybean seeds, the percent disease incidence was highest with R1 as compared to R2, R3, and R4 ((Fig. 1A and B).

Streptomyces spp ANSP4 and ANSCa22 were assessed by dual culture method for their growth inhibition against *R. bataticola* (R1 and R2) . Both demonstrated zone of inhibition in the range of 10 to 14 mm (Table 1) . These findings clearly indicate significant antagonistic potential in both the strains of *Streptomyces* against the test pathogens.

Streptomyces ANSP4 and ANSCa22 were further assessed for their efficacy for controlling R1 infection of soybean seeds by paper towel method in terms of Seedling Vigour Index (SVI). Inoculation of R1 decreased SVI by 56.01% as compared to control. SVI of R1 inoculated seeds was 666.75 which increased by 66.9% after treatment with ANSP4. Similarly remarkable increase in SVI of R1 inoculated seeds was also noted after treatment with ANSCa22. The increase in SVI of R1 inoculated seeds was greater with the biological treatment than with Carbendazim treatment where 41.84% increase was recorded (Table 2).

Antagonistic properties of *Streptomyces* were earlier reported against many fungal phytopathogens including *Alternaria brassicicola, Collectotrichum gloeosporioides, F. oxysporum, Penicillium digitatum,* and *Sclerotium rolfsii* (Khamna et. al., 2009). Control of *R. bataticola* infection by *Streptomyces* sp. was reported by Singh and Mehrotra (1982). Antagonistic activity of *Streptomyces hygroscopicus*, *Streptomyces griseoviridis* K16 and *Streptomyces lydicus* WYEC 108 were reported against *R. saloni* in independent studies (Rothrock and Gottlieb, 1984; Tahvonen and Avikainen, 1987; Yaun and Crawford, 1995).

Table.1 Antifungal activity of *Streptomyces* spp. against *R. bataticola* R1 and R2

Sr.No.	*Streptomyces* isolates	Zone of inhibition (mm)	
		R1	R2
1	ANSP4	14	12
2	ANSCa22	12	10

Table.2 Effects of different treatments on germination and seedlings growth of *R. bataticola* inoculated soybean seeds by paper towel method

S.N	Treatments	Germination percent	Average shoot length (cm)	Average root length (cm)	SVI
1	Control (C)	95%	8.81	8.416	1515.888
2	T1	99%	10.43	10.13	2014.88
3	T2	70%	4.82	4.07	666.75
4	T3	80%	7.876	6.106	1146.52
5	T4	99%	10.24	9.44	1928.64

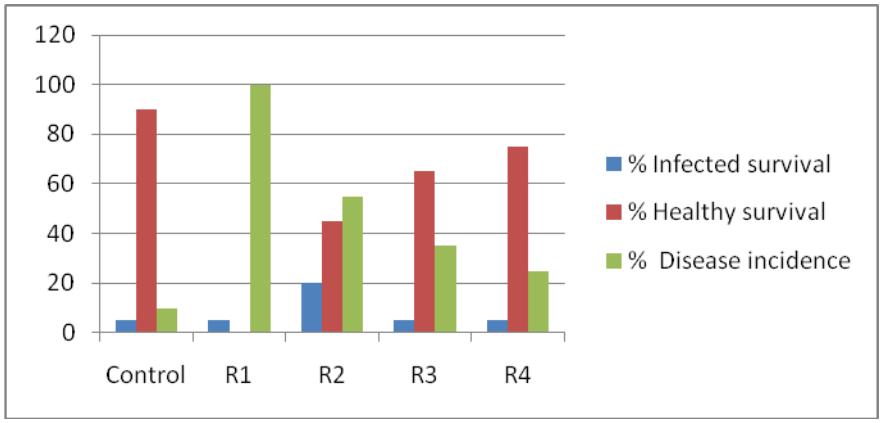
T1- Treatment with ANSP4 , T2- Pathogen control, T3- Treatment with Carbendizim , T4- Treatment with ANSCa22

Fig.1 Test of virulence of *R.bataticola* isolates for soybean seeds by sick soil method

C R2 R1 R4 R3

A-Control, B-E- Isolates of *R. bataticola*

Fig.2 Disease incidence (%) of *R. bataticola* isolates for soybean seeds



Note : R1 - R4- Isolates of *R. bataticola*

Fig.3 Effects of different treatments on germination and seedling growth of *R. bataticola* inoculated soybean seeds (A-E) by paper towel method

(A) Untreated Soybean seeds (Control C1)

(B) R1 inoculated Soybean seeds treated with ANSP4

(C) R1 inoculated Soybean seeds

(D) R1 inoculated Soybean seeds treated with Carbendizim

(E) R1 inoculated Soybean seeds treated with ANSCa22

Results of the present investigations are in accordance with such studies and revealed the antagonistic potential of *Streptomyces* ANSP4 and ANSCa22 against *R. bataticola* infection of soybean.

The most important observation with ANSP4 and ANSCa22 treatments of R1 inoculated seeds was promotion of the overall growth of seedlings as evident from their increased SVI compared with un-inoculated control (Table 1, figure A –E).The growth enhancing effect of both the *Streptomyces* strains was observed on development of both roots and shoots in terms of their average length and healthiness.

However the effect on roots appeared to be more prominent visually. The roots appeared to be healthier with numerous secondary and tertiary rootlets (Fig. 2A and 2E). In case of chemical treatment with Carbendazim the roots developed were weak with none or scanty secondary roots.

These results clearly indicate that both the *Streptomyces* isolates ANSP4 and ANSca22 are efficient not only for suppressing R1 infection but also for promotion of seedling growth of soybean. Our results are similar with many earlier reports (Doumbou et. al., 2002, Gopalkrishnana *et al.*, 2013) indicating presence of both biocontrol and plant growth promoting bioactivities in the strains of *Streptomyces* spp .

Both the isolates of *Streptomyces* spp. ANSP4 and ANSca22 were found to possess fungitoxic potential against the test pathogens R1 and R2 of *Rhizoctonia bataticola*. These isolates were effective for controlling *R. bataticola* (R1) infection of soybean and also for initial growth promotion of soybean seedlings under laboratory conditions.

Investigations regarding biochemical and enzymatic characteristics of these strains will be needed in future to understand the factors responsible for antagonism and plant growth promotion. Field trials will also be necessary in order to assess their application potential under different agro climatic conditions.

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