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Biocontrol of actinomycetes against chickpea wilt pathogen *Fusarium oxysporum* f. *sp. ciceri.*

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ABSTRACT

Wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the most destructive diseases of chickpea crop. As the pathogen survives mainly in soil, seed treatment is the viable way to control this disease. Actinomycetes are extensively distributed in natural habitat particularly crop rhizosphere and are involved in different biological and metabolic processes. Actinomyctes were isolated from rhizosheric soil samples of chickpea crop. These isolates were evaluated under *in vitro* condition against the wilt pathogen. Out of the 12 isolates, two isolates inhibited 78-80% mycelial growth of the pathogen and identified as *Streptomyces* spp.

Key words: Actinomycetes, Biocontrol, Chickpea, *Fusarium* wilt,

INTRODUCTION

Chickpea production is affected by various biotic stresses amongst which diseases caused by fungal pathogens are important one. Among fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most devastating and widespread disease of chickpea infecting the crop worldwide. It is seed and soil borne disease generally appearing in dry climates with temperature above 25^0C. Under congenial conditions the disease is reported to cause 100 per cent loss in grain yield (Landa *et al*., 2004). Due to soil borne nature once these diseases occur in the field it is difficult to manage. Seed treatment prior to sowing of crop is the best option to control the infection. However, chemical seed treatment has its own limitations as it causes soil pollution and ill effects on beneficial microorganism. Seed treatment with biocontrol agents not only control the infection of soil borne fungal pathogen but also help the host plant to mobilize and obtain macro and micro nutrients.

Actinomycetes, a group of Gram-positive, soil dwelling, filamentous bacteria are extensively distributed in natural habitat and are involved in different biological and metabolic processes (Merzaeva and Shirokikh, 2006; Gopalakrishnan *et al.,* 2011). They are reported to help in control of many soil borne diseases like *Fusarium*, *Rhizoctonia* and *Pythium* (Raaijmakers *et al.,* 2009). Considering this, during present investigation the biocontrol ability of actinomycetes isolated from rhizospheric soil was evaluated against the wilt pathogen infecting chickpea crop.

MATERIALS AND METHODS

The disease affected chickpea plants showing typical symptoms of Fusarium wilt were collected from wilt sick plot of Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri and the pathogen was isolated following standard laboratory procedure. (Pathak, 1984). The fungal colonies were purified by single hypal tip method and pure culture maintained on slants for further studies. The pathogen was identified based on the morphological characters observed under microscope.

Chickpea rhizospheric soil samples were collected from different locations of Ahmednagar district. The samples were brought to laboratory, air dried and actinomycetes were isolated using serial dilution technique. Dilutions 10^2 −10^6 (0.1ml each) were plated on starch casein agar (SCA) medium by spread plate technique and incubated at 28 ± 2^0 C for 10 days. The plates were observed for growth of actinomycete and prominent colonies were picked and further purified. Pure culture of isolates was maintained on SCA slants at 4^0C for further studies. In all 12 isolates were obtained.

These actinomycete isolates were evaluated for their antifungal activity against *Fusarium oxysporum* f. sp.*ciceri* by dual-culture assay on Glucose cassamino acid yeast extract (GCY) agar medium. Pure culture of actinomycete isolates was streaked on both the side of GCY agar plate and the fungal disc of five mm was inoculated at the centre of the plate in between the actinomycetes streaking. The plates were incubated at 28 ± 2^0 C for 10 days. The radial growth of pathogen in each plate was measured and per cent inhibition was calculated.

Out of the 12 actinomycete isolates evaluated, two isolates were found promising in inhibiting the mycelial growth of Fusarium under *in vitro* condition. To identify the isolates, morphological, cultural and physiological characteristics of these two effective actinomycete isolates were studied by adopting standard procedure. Cover slip method was used to investigate the morphological characteristics of the actinomycete isolates on SCA medium (Williams and Cross, 1971). The colony characteristics, *viz*., aerial mass colour, colour of the substrate mycelium as viewed from reverse side and diffusible colour produced were studied on five different media ISP 2, ISP3, ISP4, ISP 5 and SCA. The physiological characteristics of the isolates, *viz.* sodium chloride tolerance and ability to grow at different pH were also studied by using SCA medium.

RESULTS AND DISCUSSION

In vitro efficacy of actinomycete isolates against chickpea wilt pathogen

The fungal pathogen, i.e. *Fusarium oxysporum* f.sp.*ciceri* was isolated from the disease affected plants showing typical symptoms of wilt and it's identity was confirmed based on morphological characters of the fungus. Actinomycete isolates were isolated from rhizospheric soil samples by using serial dilution technique. The typical actinomycetes colonies developed on SCA medium from 10 days onwards were round in shape and cottony appearance. The colony characteristics of isolates are similar to those described for actinomycetes earlier by Waksman and Lechevalier (1953) and Shirling and Gottlieb (1966). The aerial mass colour and substrate mycelium colour was variable with the isolate of the actinomycete. In all, 12 isolates (ANTS-1 to ANTS-12) were obtained from different rhizospheric soil samples.

The isolates were screened for their antagonistic activity against *Fusarium oxysporum* f.sp. *ciceri* by dual culture inoculation method on Glucose Cassamino acid Yeast (GCY) extract agar medium. All the actinomycete isolates efficiently inhibited the growth of Fusarium fungus and were found significantly superior over control in inhibiting the mycelial growth (Table 1, Plate 1). Maximum inhibition (80.00 %) was obtained with isolate ANTS-5 with least colony diameter of 17.00 mm. It was followed by isolate ANTS-4 which recorded 78.82 per cent growth inhibition with 18.00 mm mean colony diameter. Thus, the two isolates i.e. ANTS-4 and ANTS-5 were significantly superior over other isolates in controlling the growth of Fusarium fungus. The per cent inhibition with rest of the isolates ranged 62.35 to 12.63.

Earlier, Gopalkrishanan *et al*. (2011) conducted study on antagonistic potential of actinomycete isolates against chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) by *in vitro* dual-culture assay and reported that among 137 actinomycete isolates, 33 had the antagonistic potential against FOC. Similarly, Ashokvadhan *et al*. (2014) reported that among 54 strains of actinomycetes, 13 strains inhibited the mycelial growth of *Fusarium oxysporum*.

Efficacy of actinomycete isolates in inhibiting the plant pathogen had also been reported by Kulkarni (2010) against *Alternaria alternata, Fusarium oxysporum* and *Rhizoctonia bataticola.* Similarly, Sussela Bhai *et al*. (2016) had reported that actinomycete isolates inhibited the growth of *Phytophthora capsici* causing foot rot and slow decline disease in black pepper. Nanjappan *et al.* (2018) and Anusha *et al.* (2019) had also reported the potential of actinomycetes to control plant pathogens.

In present studies actinomycete isolates effectively controlled the mycelial growth of Fusarium under laboratory conditions.

Morphological, colony and cultural characteristics

The cover slip method was used to investigate the morphological characters of actinomycete isolates ANTS-4 and ANTS-5. Both the isolates exhibited spiral spore arrangement. Long chain of sporophores containing more than 30-35 spores were observed and spores were cylindrical to oval in shape (Plate 2). Long chain of conidia and spore arrangement are typical as that of *Streptomyces* spp.

Different culture media like Yeast-extract-malt extract agar (ISP 2), Oatmeal agar (ISP 3), Inorganic salt-starch agar (ISP 4), Glycerol-asparagin agar (ISP 5) and Starch casein agar (SCA) were used to examine colony characterization actinomycete isolates ANTS-4 and ANTS-5. It was observed that

the growth of actinomycete isolates ANTS-4 and ANTS-5 was good on all culture media under study (Table 2 and 3). In case of isolate ANTS-4 it was observed that the colour of aerial mycelium was dark grey on ISP 2 and ISP 3, light grey on ISP 4 and ISP 5 and white on SCA. The colour of substrate mycelium was grey on ISP 2 and ISP 3, dark grey on ISP 4 and brown on ISP 5 and SCA media. There was no diffusible pigment observed. In case of actinomycete isolate ANTS-5 colour of aerial mycelium was white on ISP 2 and SCA, whitish grey on ISP 3, light grey on ISP 4, and dark grey on ISP 5. Colour of substrate mycelium was dark grey on ISP 2 and ISP 3, grey on ISP 4 and brown in ISP 5 and SCA.There was no diffusible pigment observed.

Earlier, Mythili and Das (2011) reported spiral spore arrangement in most of the actinomycete isolates and very small number of actinomycete isolates showed melanin pigment. Nanjwade *et al*. (2010) studied on the morphological and cultural characteristics of actinomycete isolates and obtain almost similar results as of present investigation.

Muleta and Assefa (2018) studied morphological and cultural characteristics of actinomycete isolates. Morphological characters were studied by cover-slip method. Most of isolates showed rectiflexible spore chain and branched mycelium. Cultural characters were studied by using different media like starch casein agar, glycerol asparagine agar, starch nitrate agar, glucose asparagine agar and actinomycetes isolation agar. Isolates grow well in all media. The colour of aerial mycelium was white to grey and substrate mycelium was gray, yellow to brown. The diffusible pigment was found only in some isolates and the diffusible colour was red.Hozzein *et al.* (2019) isolated a total of 27 actinomycete isolates from Jouf region of Saudi Arabia and studied their morphological characteristics by using cover-slip method under microscope. All isolates had different colour of aerial mycelium, substrate mycelium colour ranged from white, brown, grey to violet and spore chain were rectiflexible to verticilate.

Physiological characters

The effective actinomycete isolates ANTS-4 and ANTS-5 were studied for their physiological characters like sodium chloride tolerance and pH requirement.

Sodium chloride tolerance

From the data presented in Table 4, it is observed that the isolates ANTS-4 and ANTS-5 tolerated sodium chloride up to 5 per cent concentration and good growth of both the isolates were recorded at 0 and 5 per cent concentration of sodium chloride. At 10 per cent less growth of both actinomycete isolates was there, while at 10, 15 and 20 per cent concentration the actinomycete isolates were unable to grow and no growth was recorded.

pH requirement

In case of pH, it was observed that the actinomycete isolates ANTS-4 and ANTS-5 was able to grow on medium having pH 6 to 10, whereas the isolates showed less growth at pH 5 (Table 4). The study revealed that the actinomycete isolates can grow better at pH 6 to 10.

Almost similar findings were obtained by Umashankar *et al.* (2010) for Sodium chloride tolerance of actinomycete isolates where it was reported that sodium chloride concentration of 1.5 to 3 per cent was good for growth.Muleta and Assefa (2018) obtained similar results wherein actinomycete isolates grow well below 10 per cent concentration of sodium chloride while for pH it was observed that pH 6-10 showed good growth. Sreevidya *et al*. (2016) in their studies recorded similar observation. It was observed that the actinomycete isolate growth was good at pH6-10 and for salt tolerance the isolate tolerates NaCl concentration upto 10per cent. Similarly, Singh *et al*. (2019) in their physiological characteristic studies of actinomycete isolates reported that the isolates were able to grow at pH 6-10 and tolerated NaCl concentration up to 10 per cent.

The morphological character's exhibited by effective actinomycete isolates closely resembles with *Streptomyces* spp. as describe earlier by Waksman (1957) and Umashankar *et al.* (2010). Based on the characteristics studies the effective actinomycete isolates were identified as *Streptomyces* spp.

The actinomycete isolates thus showed promising results for Fusarium control. Wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is one of the most important and wide spread disease of chickpea crop. The disease infect crop at all stages and can cause 100 per cent yield reduction. During present studies two actinomycete isolates significantly inhibited the mycelial growth of Fusarium fungus. Further exploitation of these effective actinomycete isolates identified as *Streptomyces* spp. can play a vital role in biological control of most devastating disease of chickpea.

Table 1. *In vitro* efficacy of actinomycete isolates against *Fusarium oxysporum* f.sp. *cicero*

Sr. No.	Isolate code	Mean colony diameter (mm)*	Per cent inhibition**
1	ANTS-1	36.33	57.25 (49.16)
2	ANTS-2	40.00	52.94 (46.66)
3	ANTS-3	32.00	62.35 (52.12)
4	ANTS-4	18.00	78.82 (62.68)
5	ANTS-5	17.00	80.00 (63.41)
6	ANTS-6	62.33	26.66 (31.07)
7	ANTS-7	61.66	27.45 (31.58)
8	ANTS-8	74.26	12.63 (21.05)
9	ANTS-9	72.66	14.98 (21.65)
10	ANTS-10	25.66	66.28 (54.48)
11	ANTS-11	62.66	27.05 (30.81)
12	ANTS-12	63.33	25.88 (30.54)
13	Control	85	0.00
		S.E.±	1.03
		C.D.@ 1%	2.90

Table 2. Colony characteristics of actinomycete isolate ANTS-4

Character	Medium				
	ISP 2	ISP 3	ISP 4	ISP 5	SCA
Colour of aerial mycelium	Dark Grey	Dark Grey	Light Grey	Light Grey	White
Colour Substrate mycelium	Grey	Grey	Dark Grey	Brown	Brown
Diffusible pigment colour	-	-	-	-	-
Growth	Good	Good	Good	Good	Good

* Average of three replications; ** Figures given in parentheses are arcsine values

Table 3. Colony characteristics of actinomycete isolate ANTS-5

Character	Medium				
	ISP 2	ISP 3	ISP 4	ISP 5	SCA
Colour of aerial mycelium	White	whitish Grey	Light Grey	Dark Grey	White
Colour Substrate mycelium	Dark Grey	Dark Grey	Grey	Brown	Brown
Diffusible pigment colour	-	-	-	-	-
Growth	Good	Good	Very Good	Very Good	Very Good

Table 4. Physiological characteristics of actinomycete isolate ANTS-4 and ANTS 5

A) Sodium Chloride Tolerance limit	
0%	Good growth
5%	Good growth
10%	Less growth
15%	No growth
20%	No growth
25%	No growth
B) Ability to grow at different pH	
5	Less growth
6	Good growth
7	Good growth
8	Good growth
9	Good growth
10	Good growth

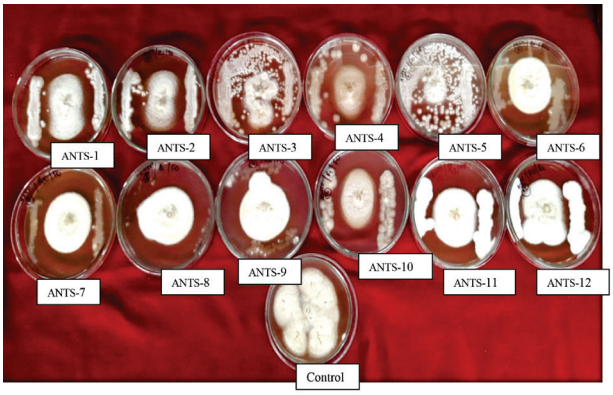

Plate 1. *In vitro* efficacy of actinomycete isolates against *Fusarium oxysporum* f. sp. *ciceri*

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