Original Research Article

Isolation, Screening and Identification of Herbicidal Actinomycetes from Rhizosphere Soils

ABSTRACT

Aim: Current study invetigates the activity of rhizosphere microflora against common weeds of *Coccinia grandis*. Soil actinomycetes known for its potential antimicrobial and other secondary metabolites were taken for the study.
Study design: CRD.
Place and Duration of Study:, Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), between November to December 2021.
Methodology: Actinomycetes were isolated from rhizosphere soils collected from western zone of Tamil Nadu (Ooty, Western Ghats, Tirupur, Erode and Coimbatore) using different media (KKA, SCA, HV, LMSA & WYEA). Their morphological characterization was by following standard protocol.The isolates were screened based on their herbicidal activity against *Trianthema portulacastrum*.*In vitro* seed germination assay (pre-emergence) was followed to identify potential actinomycetes.
Results: Thirty actinomycetes isolates were obtained from different locations of Tamil nadu using five different media and their morphological characterization was done. Most of them are creamy white, gray, white and some of them were producing pigments. All the isolates shows positive for Gram's reaction.
Conclusion: Six efficient isolates were identified in the preliminary screening of weed seed germination. Further the isolates will be studied at field level.

Keywords: weeds-Actinomycetes- herbicide-germination

1. INTRODUCTION

Weeds are undesirable plants in the crop field and caused significant economic loss in crop production. About 200 plant species in the world act as weeds, of which around 80 species are troublesome enough to humans (Holm, 1977). The composition of weed species, their intensity, and competition offered by them to the crop vary with the geographic regions, soil and weather conditions, and the field and crop management practices (Mashingaidze *et al*., 2012).

Different weed management practices were followed to control weeds. Conventional weed control methods have its own merit and demerit *viz*., manual weeding is time and labour consuming; control with herbicide having host specific/broad range limitation along with emergence of herbicide resistance. To reduce the reliance on synthetic herbicides in cropping systems bio herbicides were introduced to reduce toxic ill effect. Therefore, in recent decades, weed management has changed from conventional practices to environmentally friendly biological approaches [3].

Bio herbicides derived from microbial source are effective substances for weed control provide ecological advantages and maintain sustainable agricultural production. Secondary metabolites of identified microbial species causes plant phytotoxic activities such as necrosis, chlorosis, deformation and stunting. These features are prerequisite to use them as bio herbicides for weed control. Actinobacteria represent the most prominent group of microorganisms, which produce bioactive compounds. Majority of these molecules originate from Streptomyces genus [4].

Trianthema portulacastrum L. belongs to *Aizoaceae* family, is a common dicotyledon weed found in various agricultural and vegetable crops, especially during the rainy seasons [5]. Control of *T. portulacastrum* with various pre and post-emergence herbicides in different agricultural crops have been attempted. Pre-emergence application of oxyfluorfen, isoproturon, oxadiazon, pendimethalin and fluchloralin effectively controlled horse purslane in various crops [6,7,8].

Although these measures can control the weed on a small scale, they are not feasible for large infestations or infestations in environmentally sensitive areas. Further, increased and indiscriminate use of herbicides has resulted in herbicide resistance [9] and environmental concerns. Worldwide efforts are underway to reduce the heavy reliance on chemical herbicides and finding alternative strategies for weed management [10] is the need of time. Allelopathy and mycoherbicides could be an appropriate potential technology for this purpose.
Keeping these facts in mind, the present study was undertaken to isolate and screen actinomycetes for their herbicidal activity against Trianthema weed at pre emergence stage by microbial secondary metabolites.

2. MATERIAL AND METHODS

2.1 Sample collection and Isolation of Actinobacteria

2.1.1 Sampling sites:

The soil samples taken for this study were collected from Agricultural Fields of western zone of Tamil Nadu (Ooty, Western ghats, Tirupur, Karur & Erode) during November to December 2021. Ten gram of the collected soil samples were pre-treated in Hot Air Oven. They were heated to 60-65°C for 3 hrs. This helps in decreasing population of gram positive bacteria.

Actinobacteria were isolated from soil samples by serial-dilution and plating technique. Four different media *viz*., KKA (Ken Knights Agar), HV (Humic acid Agar), LMSA (Low Nutrient mineral salt Agar) and SCA (Starch Casein Agar) were employed for isolation of Actinobacteria with dilutions 10^{-2} and 10^{-3}. Culture medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min and supplemented with cyclohexamide antifungal agent (50µ/ml) and tetracycline antibacterial agent (20µ/ml) to prevent bacterial growth and fungal growth respectively. The plates were incubated at 28±2°C for 5 to 7 days. After incubation, the actinobacteria were observed, purified using subculture method and maintained in ISP-4 medium for further investigation.

2.2 Identification of actinobacterial isolates:

The isolated actinobacterial colonies were studied by following methods:

2.2.1 Cultural characterization

Growth on solid media:

The cultural properties and growth characteristics of isolates were studied on 7th day in ISP-4 media, the plates were incubated at 30°C and observations were recorded on the 7 th day.

Morphological Characterization:

Morphological Characterization was performed with a magnified lens and observed on actinobacterial strains grown for 3 to 14 days on ISP-4 agar media plate. Colony morphology was recorded with respect to aerial color, aerial mycelium, size, nature of colony, reverse side color and pigmentation and the isolated were observed under the microscope.

Grams staining:

A thin smear of each isolates was made on a clean glass slide and heat fixed. Then the smear was stained with crystal violet for 1 min and fixed by staining with Gram's iodine. After 30 sec the slide was washed again in tap water and decolorized with alcohol. After decolourization, the smear was counter stained with safranin for 1 min. Then the slide was washed, air dried and examined under the microscope.

2.2 Screening of actinobacterial isolates:

2.2.1 Weed seed germination assay (pre-emergence)

All the thirty isolates were purified and maintained in ISP-4 broth. Seeds of *Trianthema portucalastrum* were soaked in 3 ml of culture filtrate for 12 hours. A pertidish was taken with suitable size filter paper. Filter paper was moistened with culture filtrate. After 12 hours of soaking, seeds were placed in petridish and observed after 4 days for germination. Similar procedure without culture filtrate served as control. The experiment was repeated for 3 times as replication to confirm the herbicidal activity of all isolates. From this efficient isolates were selected and taken for further investigation.

3. RESULTS

3.1 Collection of soil samples

3.1.1 Sampling sites:

Soil samples were collected from different locations in the western zone of Tamil Nadu i.e. Ooty, Western Ghats, Tirupur, Erode and Coimbatore. Their geographical coordinates were presented in table 1.

Table 1. Geocoordinates of sampling sites

Location	Soil	Geocoordinates
Ooty	Belmounte Terrace	11.4046° N, 76.7230° E
Western ghats	Anaimalai	10.5821° N, 76.9343° E
Tirupur	Uttukuli	11.1559° N, 77.4433° E
Erode	Kodumudi	11.0788° N, 77.8867° E
Coimbatore	Pollachi	10.6609° N, 77.0048° E
	Orchard,TNAU	11.0122° N, 76.9354 E

3.2 Isolation and characterization of Actinomycetes

3.2.1 Isolation of actinobacteria:

Thirty isolates were obtained from five different locations (table 2). Soil samples from Ooty register maximum isolates (8 No.) of which 3 isolats obtained from normal soil and 5 isolates obtained from pre heated soils. Seven isolates were obtained from Coimbatore as well as Erode soils, where normal soils recorded 4 isolates from Erode and 3 isolates from Coimbatore. Pre heated soils recorded 3 isolates from Erode and 4 from Coimbatore. Tirupur soils reorded minimum number of isolates (3 Nos.) compared to other location. Here the results denotes the identically different isolates obtained from each soil not the total population of particular soil.

Table 2. Isolation of actinobacteria from different rhizosphere soil

Particulars of sample	Normal soil sample				Soil heated @ 60-62°C for 3 hrs			
	KKA	SCA	HV	LMSA	KKA	SCA	HV	LMSA
Ooty	-	-	3	-	2	2	1	-
Western Ghats	1	-	1	-	-	-	3	-
Tirupur	-	-	2	-	-	-	-	1
Erode	-	4	-	-	-	3	-	-
Coimbatore	3	-	-	-	2	-	2	-

3.2.2 Characterization of actinobacteria:

All thirty isolates were morphologically characterized based on the colony size, texture, color and pigmentation. Color of the isolates were Creamy white, white, Grey, Light yellow and Light grey. Out of 30 isolates 15 isolates had Creamy white colored colony, 8 had white color, 4 recorded grey, 1 had Light yellow, 1 had Dull white and 1 had Light grey color colony. All the isolates show pigmentation which was observed by the coloration on the reverse side of the colony. Pigmentation such as Yellowish white, Brown, Yellow, Pink, Dark greenish brown and Pink diffusible pigment. Yellowish white pigmentation was predominant among the isolates (17 isolates) followed by brown and yellow. Texture of the colony was recorde as hard for most of the isolates and few of the isolates had smooth surface.size of the colony ranges from 0.2 to 0.3 cm. All the isolates were positive for Gram's reaction (Table 3).

Table 3. Morphological characterization of actinomycetes isolates

S.No.	Strains	Color Surface	Color reverse Side	Colony texture (cm)	Colony size (cm)	Gram staining
1.	A1	Creamy white	Yellowish white	Hard	0.3	+
2.	A2	white	Yellowish white	Hard	0.2	+
3.	A3	Creamy white	Yellowish white	Hard	0.3	+
4.	A4	Creamy white	Yellowish white	Hard	0.3	+
5.	A5	white	Brown	Hard	0.2	+
6.	A6	white	Yellow	Hard	0.3	+
7.	A7	white	Yellowish white	Hard	0.3	+
8.	A8	Creamy white	Yellowish white	Hard	0.2	+
9.	A9	Creamy white	Yellowish white	Hard	0.3	+
10.	A10	Creamy white	Yellowish white	Smooth	0.3	+
11.	A11	Creamy white	Yellowish white	Smooth	0.2	+
12.	A12	white	Pink	Smooth	0.3	+
13.	A13	Grey	Brown	Hard	0.3	+
14.	A14	Grey	Yellow	Hard	0.2	+
15.	A15	Creamy white	Dark greenish Brown	Hard	0.4	+
16.	A16	Creamy white	Yellowish white	Smooth	0.2	+
17.	A17	Creamy white	Yellowish white	Hard	0.3	+

18.	A18	Dull white	Yellowish white	Hard	0.4	+
19.	A19	Grey	Yellowish white	Hard	0.3	+
20.	A20	Light yellow	Yellowish white	Hard	0.2	+
21.	A21	Creamy white	Pink diffusible Pigment	Hard	0.3	+
22.	A22	Creamy white	Yellow	Smooth	0.3	+
23.	A23	Creamy white	Brown	Smooth	0.2	+
24.	A24	White	Yellow	Hard	0.3	+
25.	A25	White	Yellowish white	Hard	0.2	+
26.	A26	Grey	Yellowish white	Hard	0.3	+
27.	A27	Light grey	Yellowish white	Hard	0.2	+
28.	A28	White	White	Smooth	0.3	+
29.	A29	Creamy white	Brown	Smooth	0.2	+
30.	A30	Creamy white	Yellow	Hard	0.3	+

3.3 Screening of actinomycetes based on herbicidal activity

3.3.1 Germination of Trianthema seeds *in vitro*

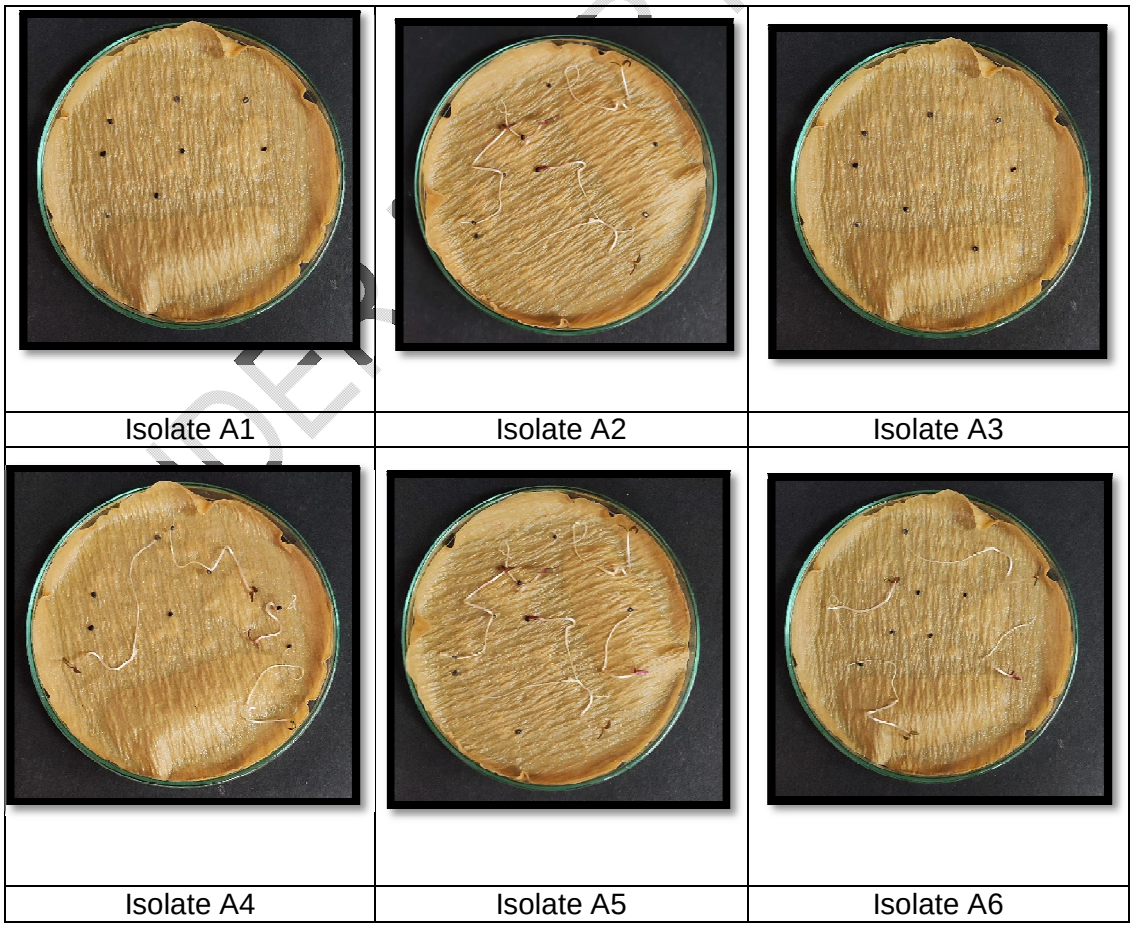
Germination of common weed of garden soil crops,*Trianathema portulacastrum* was taken for this study. Weed seeds were soaked overnight in the Culture filterate of all thirty isolates and placed in petriplate containing moistened germination paper. Based on the germination behaviour of each isolate, their herbicidal activity was determined. This was the pre-emergence control of weeds by actnomycetes isolates. There is no germination/emergence of *Trianathema* seeds in A1, A 3, A8,A12, A13, A17 isolates which denotes the herbicidal activity of the isolates against the weed seed. Isolates A15, A19, A24,A25,A28,A30 recorded 100 per cent germination, which indicates those isolates shown growth promoting activity for weed seeds. Other isolates recorded moderate growth rate for weed seeds. This results were tabulated in table 4 and plate 1.

Table 4. Germination of weed seed

Isolates	Number of seeds germinated	Germination percent (%)
Control	8	100%
A1	0	0%
A2	6	75%
A3	0	0%
A4	5	62%
A5	7	87%
A6	4	50%
A7	6	75%
A8	0	0%
A9	7	87%
A10	4	50%
A11	3	37%
A12	0	0%
A13	0	0%
A14	7	87%

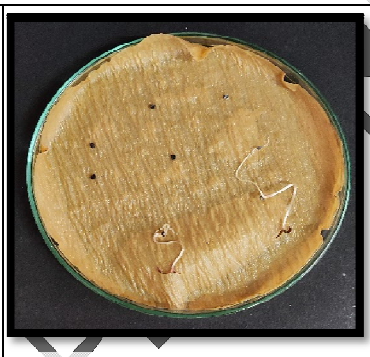
A15	8	100%
A16	4	50%
A17	0	0%
A18	6	75%
A19	8	100%
A20	4	50%
A21	5	62%
A22	6	75%
A23	4	50%
A24	8	100%
A25	8	100%
A26	6	75%
A27	7	87%
A28	8	100%
A29	7	87%
A30	8	100%

Plate 1. Effect of actnomycetes isolates on germination of trianthema



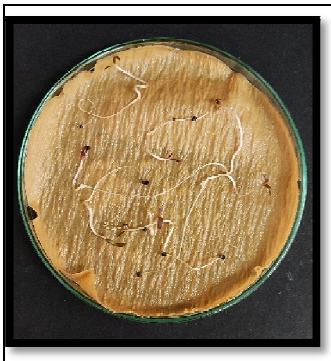
Isolate A1	Isolate A2	Isolate A3
Isolate A4	Isolate A5	Isolate A6

		
Isolate A7	Isolate A8	Isolate A9

		
Isolate A10	Isolate A11	Isolate A12
		
Isolate A 13	Isolate A 14	Isolate A 15

		
Isolate A 16	Isolate A 17	Isolate A 18

		
Isolate A 19	Isolate A20	Isolate A21
		
Isolate A22	Isolate A23	Isolate A24

		
Isolate A 25	Isolate A26	Isolate A 27

		
Isolate A 28	Isolate A 29	Isolate A30
		
	Control	

3. DISCUSSION

There is no clear cut and established method available at present for the control of this weed. however manual, chemical and biological methods are considered to control this weed. Herbicides have become one of the main environmental problems, causing long-term toxicity to water and soil resources,as well as mammals. Therefore, integrated approaches to biological weed control in arable crops along with other weed management techniques are being broadly studied. Biological control of trianthema with fungal pathogens was studied earlier by many researchers and it has gained acceptance as a practical safe and environmentally beneficial method. Earlier studies [10,11,12] indicate the potential of biological control of trianthema using plant pathogens.

Actinomycetes are important group of microorganisms producing many extracellular active compounds such as Anisomycin, Bialaphos, Herbicidin A & B [13]. Christy et al.[14] reported that a small number of microbial products combined with herbicides improved the weed control e□cacy.

In the present investigation a total of 30 actinomycetes were isolated from different rhizospheric soil samples taken from western zone of Tamil Nadu. The results of present study are comparable with the study on endophytes in which genus Streptomyces was dominant [15]. Similarly, Zhang et al. [16] reported mainly Streptomyces from medicinal plants.

In the present investigation out of 30 isolates, 6 isolates shown o per cent germination, which shows there is a 100 % inhibition of weed seed at pre emergent stage. These isolates were taken for further study. A study conducted by Helly Singh et al. (2017) [17] were isolated and screened endophytic actinomycetes for their herbicidal activity against *Parthenium hysterophorus*, *Ageratum conyzoides* and *Bidens biternata*. Direct fermentation method was used for the phytotoxin production in the submerged culture to study the herbicidal activity against different test weeds. The significant differences were observed in the production of phytoxin in SCN and GS medium. In the case of Ageratum conyzoides (billygoat weed), the culture filtrate of Nocardiodes sp. 1, Nocardiodes sp.2 and Actinomadura sp. showed 60% reduction in seed germination. These findings suggest that endophytic actinomycetes are rich source of herbicidal metabolites. Further studies are required to isolate, purify and structure elucidation of the metabolites.

4. CONCLUSION

In the present studyphytotoxic effect of rhizospheric actinomycees were tested against Trianthema weed and six effective isolates were identified with 100 % inhibition of weed seed in vitro compared to other isolates and control. These isolates were further study at field level to find out their efficiency, and their secondary metabolites will be studied to find out the active ingredient responsible for phytotoxicity.

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