In vitro evaluation of rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini* and *Rotylenchulus reniformis* infesting Castor

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ABSTRACT

A study was conducted to evaluate the efficacy of 108 rhizobacterial isolates collected from different Oilseed rhizospheres against the wilt pathogen, *Fusarium oxysporum* f.sp. *ricini* and reniform nematode, *Rotylenchulus reniformis* under *in vitro* conditions. Among the 108 isolates tested, four isolates were reported to inhibit the radial growth of *Fusarium oxysporum* f.sp. *ricini* by more than 50% and caused more than 80% of juvenile (J_4) mortality of reniform nematode. The effective rhizobacterial isolates were subjected to morphological and biochemical characterization for the assessment of plant growth promoting traits and bio-control properties.

Keywords: Castor, Fusarium oxysporum f.sp*. ricini, In vitro assay, rhizobacteria, Rotylenchulus reniformis*

1. INTRODUCTION

Castor (*Ricinus communis* L.) is a non-edible Oilseed crop grown in tropical and sub-tropical parts of the world. The crop was originated in the Ethiopian region of tropical East Africa. Non edible oil obtained from the crop seeds are used for commercial, industrial, and household purposes. In India, the crop is sown in an area of 831 thousand hectares with a production of 1619 thousand tonnes and a productivity of 1.9 tonnes ha^{-1} [1]. Approximately 25% of yield losses are caused by insects and pathogens. Among the plant pathogens, bacteria, viruses, nematodes, and fungus, seriously harm the crop worldwide and drastically lower the quantity and quality of the products, which pose a serious risk to the world's Oil supply [2].

One of the main challenges to Castor production is the disease complex formed by the reniform nematode, *Rotylenchulus reniformis*, and the wilt-inducing fungus, *Fusarium oxysporum* f. sp. *ricini*. The amount of yield loss due to disease complex varies according to the crop's stage (77% during flowering, 63% at 90 days, and 39% at later stages). Long-term survival of the pathogens in the soil and the susceptibility of common cultivars to the pathogens contribute to the accumulation of soil inoculum of the pathogen and a high incidence of wilt disease complex in the Castor-growing areas of India. The wilt disease becomes more apparent in later stages of the crop and usually appears in the flowering and spike formation stage.

Nematode infection plays a significant role in making castor wilt disease severe. Due to their predisposing nature, reniform nematodes are important plant parasitic nematodes in Castor [3]. Due to the reniform nematode's presence, castor hybrid GCH 4, which was previously resistant to the wilt disease of Andhra Pradesh, became susceptible [4]. Since both the nematode (*R. reniformis*) and the fungus (*F. oxysporum* f. sp. *ricini*) are soil-borne and may persist in the soil for extended periods of time without the host, their control mainly relies on the chemical fungicides/nematicides. Given that most chemicals are currently subjected to rigorous regulations and restrictions due to their toxicity to the environment and health concerns to humans, animals, and crops, an alternative environmentally safe method is desperately needed to control the Castor disease complex. Biopesticides based on living microorganisms are the non chemical alternatives which are eco-friendly and economically viable.

The effectiveness of *Bacillus* sp. and *Pseudomonas* sp. against nematode- fungal disease complex has been reported by several authors. Effect of *Bacillus subtilis* strain, Bbv 57 for the management of wilt - nematode disease complex caused by *Fusarium oxysporum* f. sp. *gerberae* - *Meloidogyne incognita* in Gerbera has been reported by Ramyabharathi *et al.* [5]. Similarly, the efficacy of the combined application of *B. subtilis*, Bbv 57, and *P. fluorescens*, Pfbv 22 against the disease complex induced by *Meloidogyne incognita* and *Fusarium oxysporum* in Tuberose var. Prajwal has been reported by Meena *et al.* [6]. Antagonistic activity of *B. velezensis,* Bv-DS1, against *Meloidogyne incognita* and *F. oxysporum* has been reported by Hu *et al*. [7]. Antagonistic activity of combined application of *T. viride* Trichogen-T and *P. fluorescens* Florozen-P against Castor wilt disease complex caused by *F. oxysporum* and *R. reniformis* has been reported by Shalini *et al.* [8].

The present study has been proposed to study the efficacy of native rhizobacterial isolates collected from different Oilseed rhizospheres against *Fusarium oxysporum* f.sp. *ricini* and *R. reniformis* infesting Castor.

2. MATERIALS AND METHODS

2.1 Collection of soil samples

About 108 native rhizobacterial isolates were isolated from 32 soil samples, collected from the rhizosphere of different Oilseed crops grown in and around Telangana.

2.2 Isolation of rhizosphere bacteria from soil samples

Ten g of rhizosphere soil sample was suspended in 90 ml of sterile distilled water blank and kept in a rotary shaker at 160 rpm for 30-45 min. One ml of 10^{-1} dilution thus obtained was transferred to a test tube containing 9 ml of sterile distilled water blank using a 1 ml micropipette which gives 10^{-2} dilution. This step was repeated to obtain concentrations 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively. Dilutions 10^{-6} and 10^{-7} were used for isolation of bacterial colonies. Pour plate method was followed for microbial isolation where, one ml aliquot was transferred into Petri plates to which 15 ml of sterilized and cooled nutrient agar media was added. The plates were incubated at, 28 ± 2°C for 24-48h. Then, the number of bacterial colonies were counted and sub-cultured in NA slants to obtain the pure cultures.

2.3 Source of the pathogen:

The *Fusarium* wilt pathogen associated with Castor, *Fusarium oxysporum* f.sp. *ricini* maintaining at the Division of Crop Protection, ICAR-IIOR was used for the study.

2.4 Source of the nematode:

Reniform nematode, *Rotylenchulus reniformis* culture maintaining in the glass house of Crop protection division in Castor plants was used for the study.

2.4.1 Preparation of culture filtrates of rhizobacterial isolates:

About 24 - 48h old rhizobacterial cultures grown in nutrient broth (NB) were centrifuged at 6000 rpm for 20-30 minutes [9]. The supernatant was then filtered in Whatman no.1 filter paper, which was used for *in vitro* assay against reniform nematode.

2.5 *In vitro* assays

2.5.1 *In vitro* assessment of different rhizobacterial isolates on juvenile mortality of reniform nematode

About 100µl of nematode suspension containing 100 J_4 was placed in 2 ml of culture filtrates of rhizobacterial isolates in small Petri plates. The numbers of dead juveniles were recorded at 24, 48, 72 and 96h after inoculation. Juveniles inoculated in sterilized distilled water served as control. Percentage mortality of juveniles was calculated according to formula [10].

Mortality = (Number of dead juveniles / Total juveniles) * 100

2.5.2 *In vitro* assessment of antagonistic activity of rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini*

In vitro assay of rhizobacterial isolates was conducted to determine the antagonistic activity against *F. oxysporum* by using dual culture assay [11]. The rhizobacterial isolate was streaked at one edge of PDA plate and a 5 mm mycelial disc of 5 days old *F. oxysporum* was placed at the other end and the plates were incubated at 25 ± 2℃ for 5-7 days. The plate which contains only *F. oxysporum* was served as control. The mycelial growth of the pathogen was measured in each Petri plate and expressed in millimeters (mm) from which, the per cent inhibition of mycelial growth was calculated according to Vincent [12].

$$I = [(C - T)/C] * 100$$

where I = Per cent growth inhibition
C = Radial growth of the pathogen in control (mm)
T = Radial growth of the pathogen in treatment (mm)

2.6 Morphological characteristics of rhizobacterial isolates

The effective rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini* and *R. reniformis* were morphologically characterized.

2.6.1 Colony morphology

A single colony of isolated, purified bacteria was streaked over NA medium and incubated for 72 hours at 28 ± 2°C. Following incubation, the colonies were observed for the various morphological characteristics, including shape, size, colour, margin and colony appearance.

2.6.2 Gram staining

To the clean, grease free glass slide, a thin bacterial smear was added and air dried and heat fixed by passing over a flame. A drop of crystal violet was poured and kept for about 30 seconds and rinsed with water. Then, the slide was flooded with Gram's iodine for one minute and washed with distilled water. The slide was discoloured by adding 95% alcohol drop by drop, until the entire colour was removed. Then, the slide was counterstained with safranin for about 30 seconds and washed with distilled water. Finally, slide was air dried and observed under the microscope. The bacteria which has taken red colour were classified as gram-negative and which retained blue colour were classified as gram-positive [13].

2.7 Biochemical characteristics of rhizobacterial isolates

The effective rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini* and *R.reniformis* have been tested for different biochemical parameters *viz.*, KOH, Oxidase, Gelatinase, Catalase, Siderophores, Hydrogen cyanide, Ammonia, Organic acid, Protease, Indole, Methyl red, Voges Proskauer test, Citrate utilization, Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose and Sucrose by using standard protocols [14].

2.8 Statistical analysis

Data were subjected to analysis of variance (ANOVA) and data pertaining to percentages were angular transformed. Statistical analysis was carried out as per the procedures outlined by Gomez and Gomez [15] and Panse and Sukhatme [16]**.**

3. RESULTS AND DISCUSSION

The present research was aimed to find out the most potential rhizobacterial isolate against *Fusarium oxysporium* f.sp. *ricini* and reniform nematode, *Rotylenchulus reniformis* under *in vitro* conditions.

3.1 *In vitro* effect of rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini*

Antagonistic activity of 108 rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini* was studied by using dual culture assay. In the present study, per cent inhibition of the rhizobacterial isolates against *F. oxysporum* f.sp. *ricini* was ranged between 31- 76%. Among all the rhizobacterial isolates, maximum inhibition percent was recorded by RB65 (76.46%) followed by RB38 (61.05%), RB94 (53.44%) and RB72 (52.15%) (Table 1, Fig.1).

The results of the present study coincides with earlier work of Chowhan *et al.* [17] who identified the antagonistic activity of bacterial isolates isolated from forest soils of Chintoor, East Godavari district, Andhra Pradesh and observed 26-79% inhibition against *F. oxysporum*.

Suthar *et al.* [18] isolated 14 potential antagonist bacteria from rhizospheric soil samples of healthy chickpea and identified 3 potential antagonistic bacteria, *Burkholderia* spp. (K10), *B. subtilis* (K18) and *B. aryabhattai* (K21) with very prominent antagonistic activity of 21.7 to 75.0% against *F. oxysporum*.

Table 1. *In vitro* antagonistic activity of rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini*

S.No.	Isolates	Radial growth of *F. oxysporum* f.sp. *ricini* (mm)	Per cent inhibition of radial growth over control
1	RB1	52.44 (46.38)	41.73 (40.22)
2	RB2	55.79 (48.30)	38.00 (38.04)
3	RB3	60.21 (50.87)	33.10 (35.10)
4	RB4	51.02 (45.56)	43.30 (41.13)
5	RB5	56.44 (48.68)	37.28 (37.61)
6	RB6	46.47 (42.95)	48.36 (44.04)
7	RB7	48.71 (44.24)	45.87 (42.61)
8	RB8	60.95 (51.37)	32.27 (34.6)
9	RB9	46.61 (43.03)	48.21 (43.95)
10	RB10	57.28 (49.16)	36.34 (37.06)
11	RB11	55.77 (48.29)	38.02 (38.05)
12	RB12	51.93 (46.08)	42.29 (40.54)
13	RB13	49.23 (44.54)	45.29 (42.28)
14	RB14	58.49 (49.87)	35.00 (36.25)
15	RB15	54.87 (47.77)	39.03 (38.64)
16	RB16	51.35 (45.75)	42.93 (40.92)
17	RB17	58.09 (49.68)	35.44 (36.52)
18	RB18	51.22 (45.68)	43.08 (41.01)
19	RB19	59.52 (50.47)	33.86 (35.56)
20	RB20	52.55 (46.44)	41.61 (40.15)
21	RB21	51.60 (45.89)	42.66 (40.76)
22	RB22	57.03 (49.02)	36.62 (37.22)
23	RB23	61.56 (51.66)	31.59 (34.18)
24	RB24	56.06 (48.46)	37.70 (37.86)
25	RB25	46.37 (42.90)	48.47 (44.1)
26	RB26	56.48 (48.70)	37.23 (37.58)
27	RB27	54.82 (47.74)	39.08 (38.67)
28	RB28	62.11 (51.99)	30.98 (33.80)
29	RB29	55.11 (47.91)	38.76 (38.48)
30	RB30	60.36 (50.96)	32.93 (35.00)
31	RB31	60.88 (51.26)	32.34 (34.64)

32	RB32	47.85 (43.75)	46.82 (43.16)
33	RB33	50.94 (45.52)	43.40 (41.18)
34	RB34	52.73 (46.57)	41.40 (40.03)
35	RB35	55.44 (48.10)	38.39 (38.27)
36	RB36	57.33 (49.19)	36.29(37.02)
37	RB37	52.70 (46.53)	41.44 (40.05)
38	RB38	35.04 (36.27)	61.05 (51.36)
39	RB39	47.21 (43.38)	47.54 (43.57)
40	RB40	47.86 (43.75)	46.81 (43.15)
41	RB41	51.66 (45.93)	42.59 (40.72)
42	RB42	53.47 (46.97)	40.58 (39.55)
43	RB43	48.27 (43.99)	46.36 (42.89)
44	RB44	56.19 (48.53)	37.56 (37.78)
45	RB45	59.12 (50.23)	34.30 (35.83)
46	RB46	53.15 (46.78)	40.94 (39.76)
47	RB47	60.64 (51.15)	32.61 (34.81)
48	RB48	61.53 (51.64)	31.62 (34.2)
49	RB49	52.67 (46.51)	41.47 (40.07)
50	RB50	51.92 (46.08)	42.31 (40.56)
51	RB51	47.37 (43.47)	47.36 (43.46)
52	RB52	51.48 (45.83)	42.79 (40.83)
53	RB53	59.17 (50.26)	34.25 (35.8)
54	RB54	57.13 (49.08)	36.51 (37.16)
55	RB55	61.70 (51.74)	31.44 (34.09)
56	RB56	59.65 (50.54)	33.71 (35.48)
57	RB57	55.21 (47.97)	38.65 (38.42)
58	RB58	61.39 (51.56)	31.78 (34.29)
59	RB59	52.60 (46.47)	41.54 (40.11)
60	RB60	56.24 (48.56)	37.50 (37.74)
61	RB61	61.74 (51.77)	31.39 (34.06)
62	RB62	52.73 (46.54)	41.40 (40.03)
63	RB63	51.64 (45.92)	42.61 (40.73)
64	RB64	58.00 (49.58)	35.55 (36.58)
65	RB65	21.18 (27.37)	76.46 (60.95)

66	RB66	60.48 (51.03)	32.79 (34.91)
67	RB67	59.94 (50.71)	33.39 (35.28)
68	RB68	48.92 (44.36)	45.64 (42.48)
69	RB69	47.95 (43.80)	46.71 (43.09)
70	RB70	48.84 (44.31)	45.72 (42.52)
71	RB71	47.30 (43.43)	47.43 (43.51)
72	RB72	43.09 (41.0)	52.12 (46.19)
73	RB73	61.95 (51.89)	31.16 (33.91)
74	RB74	59.45 (50.42)	33.94 (35.61)
75	RB75	58.22 (49.71)	35.30 (36.43)
76	RB76	58.52 (49.88)	34.97 (36.23)
77	RB77	56.69 (48.82)	37.00 (37.45)
78	RB78	52.38 (46.34)	41.79 (40.26)
79	RB79	51.45 (45.81)	42.82 (40.85)
80	RB80	60.51 (51.04)	32.76 (34.89)
81	RB81	59.16 (50.26)	34.26 (35.8)
82	RB82	59.68 (50.56)	33.68 (35.46)
83	RB83	61.65 (51.71)	31.49 (34.12)
84	RB84	47.33 (43.45)	47.40 (43.49)
85	RB85	52.54 (46.43)	41.61 (40.15)
86	RB86	54.31 (47.45)	39.65 (39.01)
87	RB87	57.27 (49.16)	36.35 (37.06)
88	RB88	57.68 (49.40)	35.90 (36.79)
89	RB89	50.77 (45.42)	43.58 (41.29)
90	RB90	48.08 (43.88)	46.57 (43.02)
91	RB91	52.44 (46.38)	41.72 (40.21)
92	RB92	53.53 (47.00)	40.51 (39.51)
93	RB93	55.31 (48.03)	38.54 (38.35)
94	RB94	41.90 (40.32)	53.44 (46.95)
95	RB95	49.77 (44.85)	44.69 (41.93)
96	RB96	51.94 (46.09)	42.28 (40.54)
97	RB97	57.14 (49.08)	36.50 (37.15)
98	RB98	58.72 (50.00)	34.74 (36.10)
99	RB99	52.57 (46.45)	41.58 (40.13)

100	RB100	60.45 (51.01)	32.83 (34.94)
101	RB101	51.93 (46.08)	42.29 (40.55)
102	RB102	56.24 (48.56)	37.50 (37.74)
103	RB103	59.17 (50.26)	34.25 (35.8)
104	RB104	57.91 (49.53)	35.65 (36.63)
105	RB105	51.60 (45.89)	42.65 (40.76)
106	RB106	57.91 (49.53)	35.65 (36.64)
107	RB107	55.12 (47.92)	38.74 (38.48)
108	RB108	53.01 (46.70)	41.09 (39.85)
109	Control	90.00 (71.53)	0.00 (0.00)
110	C.D	1.61	1.05
111	SE(m)	0.57	0.37
112	SE(d)	0.81	0.53
113	C.V	2.10	1.68

Values expressed are mean of three replications;
Figures in the parenthesis are arc sine transformed values.

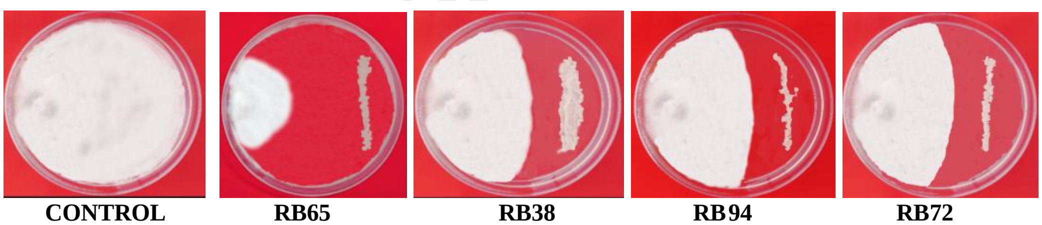


Fig. 1. *In vitro* antagonistic effect of rhizobacterial isolates against *Fusarium oxysporum* f.sp. *ricini*

Where, RB=Rhizobacteria

3.2 In vitro effect of different rhizobacterial isolates on juvenile mortality of reniform nematode

Effect of 108 rhizobacterial isolates on juvenile mortality of reniform nematode was recorded under in vitro conditions. Among the different rhizobacterial isolates tested, maximum juvenile mortality was recorded by the isolates RB65 (97.0%) followed by, RB72 (96.0%), RB38 (89.0%) and RB94 (83.0%) at 96h post inoculation (Table 2).

The results of the present study are in similarity with Swathi *et al.* [19] who isolated four native bacterial antagonists of reniform nematode, *Rotylenchulus reniformis* from rhizosphere of Cowpea and observed the juvenile mortality of 98.50 to 100.0% at 24h post inoculation under *in vitro* conditions. The authors designated *Lysinibacillus capsici*, *B. thuringiensis*, and *B. paramycoides* as the potential bacteria against *R. reniformis*. The efficacy of *Pseudomonas fluorescens* on *Meloidogyne incognita* has been proved by Meena *et al.* [20].

Table 2. *In vitro* effect of rhizobacterial isolates on juvenile mortality of *R. reniformis*

S.No	Isolate	Juvenile mortality (%)			
		24h	**48h**	**72h**	**96h**
1	RB1	15.66 (23.30)	49.33 (44.60)	65.33 (49.33)	73.00 (58.67)
2	RB2	14.33 (22.23)	47.66 (43.64)	62.33 (47.66)	71.33 (57.60)
3	RB3	15.33 (23.04)	45.33 (42.30)	60.66 (45.33)	73.66 (59.10)
4	RB4	12.66 (20.83)	50.00 (44.98)	60.66 (50.00)	70.66 (57.18)
5	RB5	11.33 (19.66)	47.00 (53.90)	59.00 (47.00)	77.00 (61.32)
6	RB6	11.33 (19.66)	42.66 (40.76)	67.66 (42.66)	74.00 (59.32)
7	RB7	16.66 (24.08)	41.33 (39.99)	68.33 (41.33)	71.00 (57.39)
8	RB8	16.66 (24.08)	41.00 (39.79)	66.66 (41.00)	71.33 (57.60)
9	RB9	15.33 (23.04)	41.00 (39.79)	63.00 (41.00)	76.00 (60.64)
10	RB10	11.66 (19.96)	41.00 (39.79)	62.00 (41.00)	76.66 (61.09)
11	RB11	11.66 (19.96)	43.33 (41.15)	71.33 (43.33)	76.66 (61.09)
12	RB12	12.33 (20.54)	41.33 (39.99)	70.33 (41.33)	78.00 (62.00)
13	RB13	15.33 (23.04)	42.66 (40.76)	71.66 (42.66)	78.33 (62.23)
14	RB14	15.33	40.00	72.00	79.33

		(23.04)	(39.21)	(40.00)	(62.93)
15	RB15	12.33	39.66	68.33	77.66
		(20.54)	(39.02)	(39.66)	(61.77)
16	RB16	12.00	47.66	66.66	76.66
		(20.24)	(43.64)	(47.66)	(61.09)
17	RB17	12.66	45.33	63.00	73.00
		(20.82)	(42.30)	(45.33)	(58.67)
18	RB18	11.66	50.00	62.00	73.00
		(19.95)	(44.98)	(50.00)	(58.67)
19	RB19	13.66	47.00	71.33	72.66
		(21.68)	(43.26)	(47.00)	(58.45)
20	RB20	11.66	42.66	68.33	74.33
		(19.96)	(40.76)	(42.66)	(59.53)
21	RB21	12.66	41.33	66.66	80.33
		(20.83)	(39.99)	(41.33)	(63.65)
22	RB22	14.33	41.00	63.00	78.00
		(22.23)	(39.79)	(41.00)	(62.00)
23	RB23	11.66	38.00	62.00	79.00
		(19.96)	(38.04)	(38.00)	(62.70)
24	RB24	11.66	33.66	71.33	77.00
		(19.96)	(35.45)	(33.66)	(61.32)
25	RB25	15.66	36.33	60.66	77.00
		(23.30)	(37.05)	(36.33)	(61.32)
26	RB26	15.66	40.33	60.66	79.00
		(23.30)	(39.41)	(40.33)	(62.70)
27	RB27	14.33	42.33	59.00	79.00
		(22.23)	(40.57)	(42.33)	(62.70)
28	RB28	11.66	41.00	67.66	76.00
		(19.95)	(39.79)	(41.00)	(60.64)
29	RB29	16.33	41.00	68.33	74.00
		(23.82)	(39.79)	(41.00)	(59.32)
30	RB30	11.33	43.33	66.66	75.66
		(19.66)	(41.15)	(43.33)	(60.42)
31	RB31	17.66	41.33	63.00	73.00
		(24.84)	(39.99)	(41.33)	(58.67)

32	RB32	12.667 (20.83)	42.66 (40.76)	62.00 (42.66)	76.66 (61.09)
33	RB33	11.66 (19.95)	40.00 (39.21)	71.33 (40.00)	78.00 (62.00)
34	RB34	16.33 (23.82)	39.66 (39.02)	70.33 (39.66)	78.33 (62.25)
35	RB35	11.33 (19.61)	42.66 (40.76)	71.66 (42.67)	79.33 (62.93)
36	RB36	17.66 (24.84)	48.00 (43.83)	72.00 (48.00)	77.66 (61.77)
37	RB37	12.66 (20.83)	45.66 (42.49)	67.66 (45.66)	75.00 (59.97)
38	RB38	23.00 (28.64)	51.66 (45.93)	82.00 (51.66)	89.00 (70.61)
39	RB39	15.66 (23.30)	41.00 (39.79)	65.33 (41.00)	73.00 (58.67)
40	RB40	14.33 (22.23)	41.00 (39.79)	62.33 (41.00)	71.33 (57.60)
41	RB41	15.33 (23.04)	43.33 (41.15)	60.66 (43.33)	73.66 (59.10)
42	RB42	12.66 (20.83)	41.33 (39.99)	60.66 (41.33)	70.66 (57.18)
43	RB43	11.33 (19.66)	42.66 (40.76)	59.00 (42.66)	77.00 (61.32)
44	RB44	11.33 (19.66)	40.00 (39.21)	67.66 (40)	74.00 (59.32)
45	RB45	16.66 (24.08)	39.66 (39.02)	68.33 (39.66)	71.00 (57.39)
46	RB46	16.66 (24.08)	49.33 (44.6)	66.66 (49.333)	71.33 (57.606)
47	RB47	15.33 (23.04)	47.66 (43.64)	63.00 (47.66)	76.00 (60.64)
48	RB48	11.66 (19.96)	45.33 (42.30)	62.00 (45.33)	76.66 (61.02)
49	RB49	11.66	50.00	71.33	73.00

		(19.96)	(44.98)	(50.00)	(58.67)
50	RB50	11.33 (19.66)	47.00 (43.22)	70.33 (47.00)	73.00 (58.67)
51	RB51	17.66 (24.84)	42.66 (40.76)	71.66 (42.67)	72.66 (58.456)
52	RB52	10.66 (19.05)	41.33 (39.99)	72.00 (41.33)	74.33 (59.53)
53	RB53	17.66 (24.83)	41.00 (39.79)	68.33 (41.00)	80.33 (63.65)
54	RB54	13.66 (21.67)	41.00 (39.79)	66.66 (41.00)	78 (62.07)
55	RB55	17.33 (24.59)	41.00 (39.79)	63.00 (41.00)	79.00 (62.70)
56	RB56	11.66 (19.95)	43.33 (41.15)	62.00 (43.33)	77.00 (61.31)
57	RB57	15.66 (23.30)	41.33 (39.99)	71.33 (41.33)	77.00 (61.32)
58	RB58	15.66 (23.30)	42.66 (40.76)	68.33 (42.66)	79.00 (62.70)
59	RB59	14.33 (22.23)	40.00 (39.21)	66.66 (40.00)	77.00 (61.32)
60	RB60	15.33 (23.04)	39.66 (39.02)	63.00 (39.66)	74.00 (59.32)
61	RB61	12.66 (20.83)	45.66 (42.49)	62.00 (45.66)	78.33 (62.23)
62	RB62	11.33 (19.66)	42.00 (40.38)	71.33 (42.00)	79.33 (62.93)
63	RB63	11.33 (19.66)	43.33 (41.15)	67.33 (43.33)	72.00 (58.03)
64	RB64	16.66 (24.08)	38.66 (38.43)	64.33 (38.66)	69.66 (56.55)
65	RB65	26.33 (30.86)	49.00 (44.49)	88.00 (49.00)	97.00 (80.08)
66	RB66	15.33 (23.04)	49.33 (44.6)	71.66 (49.33)	76.66 (61.09)

67	RB67	11.66 (19.96	47.66 (43.64)	72.33 (47.66)	78.00 (62.00)
68	RB68	11.66 (19.96	45.33 (42.30)	69.00 (45.33)	78.33 (62.23)
69	RB69	15.33 (23.04)	50.00 (44.98)	71.00 (50.00)	79.33 (62.93)
70	RB70	14.66 (22.49)	47.00 (43.26)	69.00 (47.00)	77.66 (61.77)
71	RB71	10.66 (19.03)	42.66 (40.76)	70.33 (42.66)	74.00 (59.32)
72	RB72	20.66 (27.01)	41.33 (39.99)	84.00 (41.33)	96.00 (78.41)
73	RB73	15.33 (23.01)	41.00 (39.79)	65.33 (41.00)	73.00 (58.67)
74	RB74	11.66 (19.96)	41.00 (39.79)	62.33 (41.00)	71.33 (57.60)
75	RB75	11.66 (19.96)	41.00 (39.79)	60.66 (41.00)	73.66 (59.13)
76	RB76	15.33 (23.01)	43.33 (41.15)	60.66 (43.33)	70.66 (57.18)
77	RB77	11.33 (19.66)	41.33 (39.99)	59.00 (41.33)	77.00 (61.31)
78	RB78	12.00 (20.24)	42.66 (40.76)	67.66 (42.66)	74.00 (59.32)
79	RB79	11.66 (19.95)	40.00 (39.21)	68.33 (40.00)	71.00 (57.39)
80	RB80	15.33 (23.04)	39.66 (39.02)	66.66 (39.66)	71.33 (57.60)
81	RB81	11.66 (19.96)	41.00 (39.79)	63.00 (41.00)	76.00 (60.64)
82	RB82	11.66 (19.96)	41.00 (39.79)	62.00 (41.00)	76.66 (61.09)
83	RB83	15.33 (23.04)	43.33 (41.15)	60.66 (43.333)	72.00 (58.031)
84	RB84	10.66	41.33	59.00	72.33

		(19.05)	(39.99)	(41.33)	(58.24)
85	RB85	13.66	42.66	67.66	70.66
		(21.68)	(40.76)	(42.66)	(57.15)
86	RB86	10.33	40.00	68.33	69.66
		(18.73)	(39.21)	(40)	(56.55)
87	RB87	9.66	39.66	66.66	72.33
		(18.10)	(39.02)	(39.67)	(58.24)
88	RB88	13.66	41.00	63.00	77.66
		(21.68)	(39.79)	(41)	(61.77)
89	RB89	11.66	41.00	62.00	76.66
		(19.96)	(39.79)	(41)	(61.09)
90	RB90	15.33	43.33	76.00	78.00
		(23.04)	(41.15)	(43.33)	(62.07)
91	RB91	11.66	41.33	72.00	78.33
		(19.96)	(39.99)	(41.33)	(62.23)
92	RB92	11.66	42.66	70.33	79.33
		(19.96)	(40.76)	(42.66)	(62.93)
93	RB93	15.33	40.00	68.00	77.66
		(23.04)	(39.21)	(40.00)	(61.77)
94	RB94	19.00	39.66	78.00	83.00
		(25.82)	(39.02)	(39.66)	(65.62)
95	RB95	13.33	49.33	65.33	73.00
		(21.40)	(44.60)	(49.33)	(58.67)
96	RB96	17.33	47.66	62.33	71.33
		(24.59)	(43.64)	(47.66)	(57.60)
97	RB97	11.66	45.33	60.66	73.66
		(19.96)	(42.30)	(45.33)	(59.10)
98	RB98	11.33	50.00	60.66	70.66
		(19.66)	(44.98)	(50.00)	(57.18)
99	RB99	10.66	47.00	59.00	77.00
		(19.03)	(43.26)	(47.00)	(61.32)
100	RB100	13.33	42.66	67.66	74.00
		(21.40)	(40.76)	(42.66)	(59.32)
101	RB101	11.33	41.33	68.33	71.00
		(19.66)	(39.99)	(41.33)	(57.39)

102	RB102	17.66 (24.84)	41.00 (39.79)	66.66 (41.00)	71.33 (57.66)
103	RB103	10.66 (19.05)	41.00 (39.79)	63.00 (41.00)	76.00 (60.64)
104	RB104	17.66 (24.83)	43.33 (41.152	62.00 (43.33)	76.66 (61.09)
105	RB105	13.66 (21.67)	41.33 (39.99)	71.33 (41.33)	78.00 (62.00)
106	RB106	10.33 (18.73)	42.66 (40.76)	70.33 (42.66)	78.33 (62.23)
107	RB107	13.33 (21.40)	40.00 (39.21)	71.66 (40.00)	79.33 (62.93)
108	RB108	11.33 (19.66)	39.66 (39.0)	72.00 (39.66)	77.66 (61.77)
109	Control	0.00	0.00	0.00	0.00
110	C.D	0.94	0.84	0.96	0.93
111	SE(m)	0.33	0.30	0.34	0.33
112	SE(d)	0.48	0.42	0.49	0.47
113	C.V	2.73	1.29	1.10	0.96

Values expressed are mean of three replications;
Figures in the parenthesis are arc sine transformed values.

3.3 Morphological characteristics of effective rhizobacterial isolates

The effective four bacterial colonies (RB38, RB65, RB72, and RB94) were observed for morphological traits such as gram reaction, shape, margin, elevation, size, colour, optical property, appearance and shape of the cell. The isolates were subjected to gram staining and found that RB94 as gram negative and RB38, RB65, and RB72 as gram positive in reaction (Table 3).

Suman *et al*. [21] isolated bacterial isolates from rice rhizosphere and characterized for their morphological characters. The authors identified the colonies as small to medium, smooth, glistening, convex elevation and gram negative and identified as *Pseudomonas* sp. In the present study, one effective isolate was reported with small, smooth, glistening colonies and convex elevation.

Saad *et al*. [22] isolated morphologically distinct bacterial isolates from tree rhizosphere. Colonies with morphological characters having white colour, irregular shape with flat elevation were identified as *Bacillus* spp. In the present study also three isolates showed white or dull white in colour having irregular shape and flat elevation indicating that these isolates belong to *Bacillus* sp.

Table 3. Morphological characteristics of rhizobacterial isolates

Isolate	Shape	Margin	Elevation	Size	Colour	Optical property	Appearance	Gram stain	Cell shape
RB38	Rhizoid	Lobate	Flat	Medium	Dull White	Opaque	Smooth	Positive	Rod
RB65	Irregular	Undulate	Flat	Medium	White	Opaque	Wrinkled	Positive	Rod
RB72	Irregular	Undulate	Flat	Medium	Dull White	Opaque	Wrinkled	Positive	Rod
RB94	Circular	Entire	Convex	Small	Cream	Opaque	Smooth, glistening	Negative	Rod

3.4 Biochemical characteristics of effective rhizobacterial isolates

Biochemical tests like Indole, Methyl red, Voges Proskauer test, Citrate utilization, Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose, Sucrose, KOH, Oxidase, Gelatinase, Catalase, Siderophores, Hydrogen cyanide, Ammonia, Organic acid and Protease were carried out for identification of the four potential rhizobacterial isolates (Table 4, Fig. 2).

In Indole production test, catalase and oxidase all the four bacterial isolates RB38, RB65, RB72 and RB94 were shown positive (+). Ammonia production is related to the biological control efficiency of the bacteria [23]. The three isolates RB38, RB72 and RB65 showed moderately positive and RB94 showed highly positive result for ammonia production. A study conducted by Suja *et al.* [24] reported 83 % of the tested bacterial isolates had the capability to produce ammonia, which is considered a common trait among these bacteria and indirectly influences plant growth. Plants, on their own, cannot directly absorb ammonia. However, microorganisms have developed biological nitrogen fixation mechanisms through which they can make ammonia available to plants. Therefore, the presence of these ammonia producing microorganisms can significantly enhance the availability of nitrogen to plants, leading to improved growth and ultimately higher crop yields.

HCN is produced by many rhizobacteria and is known to play a major role in biocontrol of pathogens [25]. The isolates RB38, RB72, RB94 isolates reported moderately positive while isolate RB65 showed positive for HCN production. The above studies were in agreement with the result of Syed *et al.* [26] who reported that among all the tested isolates KNL-1 (*Bacillus subtilis* strain JMP-B) and ANT-4 (*Bacillus subtilis* strain SBMP4) tested positive for HCN production.

Siderophore produced by bacteria plays a major role against pathogenic microbes in iron limitation. The isolates RB65 and RB72 showed moderately positive, isolate RB94 showed highly positive, RB38 negative in siderophore production. Kotasthane *et al.* [27] evaluated 29 isolates of *Pseudomonas* for siderophore production and reported that all isolates exhibited an orange halo after 3 days of incubation on CAS agar plate. Siderophores, iron-chelating compounds produced by microbes, play a crucial role in providing iron to plants through root-based chelate breakdown or ligand exchange, thereby promoting plant growth [28]. Additionally, these siderophores act as natural inhibitors of soil-borne fungi by reducing the availability of ferric ions to rhizosphere microflora [29].

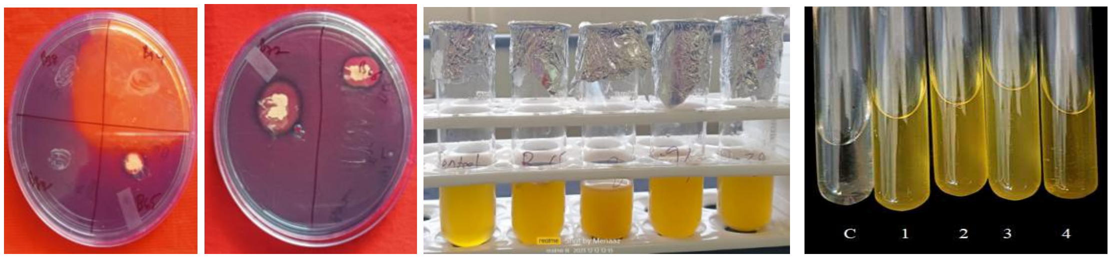
The present study is in agreement with Ramyabharathi *et al.* [30] who reported that Phenotypic characterizations of bacterial isolates were carried out to study the basic characters viz., gram reaction and utilization of different carbon sources. *B. subtilis* Bbv57, RBG1 and all the isolates were Gram positive, spore forming bacteria in the presence of 7% NaCl. They utilize citrate as sole carbon source. They were catalase positive, efficiently hydrolyzed starch and gelatin. The result obtained by analyzing primary character and carbon source utilization of different rhizosphere bacteria revealed that they belong to *Bacillus* sp. Similarly Tilak and Srinivasa Reddy [31] identified isolates from maize as *Bacillus cereus* and *B. circulans* by biochemical characteristics and profile of fatty acids.

Table 4 Biochemical characteristics of rhizobacterial isolates

S.no	Biochemical test	Effective Rhizobacterial isolates			
		RB38	RB65	RB72	RB94
1	Indole	+	+	+	+
2	Methyl Red	-	-	-	-
3	Voges Proskauer Test	-	+	-	-
4	Citrate utilization	+	+	+	-
5	Glucose	+	+	-	-
6	Adonitol	-	-	-	-
7	Arabinose	-	-	-	-
8	lactose	-	-	-	-
9	Sorbital	-	-	-	+
10	Mannitol	+	+	-	-
11	Rhamnose	-	-	-	+
12	Sucrose	+	-	-	-
13	KOH	+	+	+	+
14	Oxidase	+	+	+	+
15	Gelatinase	+	+	+	+
16	Catalase	+	+	+	+
17	Siderophores	-	++	++	+++
18	HCN	+	++	+	+

19	Ammonia	++	++	++	+++
20	Organic acid	+	++	+	-
21	Protease	+	+	++	-

-: negative; +: slightly positive; ++: moderately positive; +++: highly positive



A. Siderophore production ***B. Gelatinase test*** ***C. Ammonia production***

(C- CONTROL, 1- RB38, 2-RB65, 3-RB72, 4-RB94)

Fig.2. Biochemical characterization of effective rhizobacterial isolates

4. CONCLUSION

In the present study, among the 108 rhizobacterial isolates tested, four isolates exhibited more than 50% inhibition of *Fusarium oxysporum* f.sp. *ricini* and more than 80% juvenile mortality of reniform nematode under *in vitro* conditions. Among all the isolates, RB65 was observed to be highly effective against *Fusarium oxysporum* f.sp. *ricini* and *R. reniformis*. The isolate was confirmed as *Bacillus* sp. through morphological and biochemical characterization. Further studies are required to validate its effectiveness under pot and field conditions against *F. oxysporium* f.sp. *ricini* and *R. reniformis* and to characterize the antifungal and nematicidal compounds produced by these rhizobacterial isolates.

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