



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2022; 11(9): 2161-2166  
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Received: 19-07-2022

Accepted: 24-08-2022

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## *In vitro* efficacy of selected potential bioagents against soil borne pathogen *Sclerotium rolfsii* Sacc.

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### Abstract

The Banyan (*Ficus benghalensis*) is the National tree of India which belongs to Family Moraceae. It is used as the medicinal as well as ornamental plant and is also called as Vad, Bargad and Banyan in India. In some plants rhizospheric activities stimulate the microflora which helps in disease management as well as plant development. Present investigations were conducted to study *in vitro* antagonistic ability of the microflora isolated from the rhizospheric soil of banyan tree against *Sclerotium rolfsii*. The antifungal activity against *Sclerotium rolfsii* treatment T<sub>8</sub> (*Pseudomonas striata*) found most effective showed highest zone of inhibition i.e. 74.44%. On comparison with bioagents of VNMKV Parbhani, isolated strains of *Pseudomonas fluorescense* (73.25%), *Pseudomonas striata* (73.92%) and *Bacillus subtilis* (74.44%) are better over the available strains of VNMKV i.e. *Pseudomonas fluorescense* (69.62%), *Pseudomonas striata* (72.84%) and *Bacillus subtilis* (72.21%) respectively. The isolated *Trichoderma koningi* (68.51%) was at par to the available strains of VNMKV *Trichoderma koningi* (68.51%).

**Keywords:** *In vitro*, antagonists, *Pythium aphanidermatum*

### Introduction

In Indian tradition *Ficus benghalensis* L. (Banyan tree), *Ficus religiosa* L. (Pipal tree) and *Ficus recemosa* L. (Umbar tree) are worshiped because each and every part these trees is useful in human life. From the reviews it was observed that the rhizospheric soil contains huge number of beneficial microflora which helps these plants for their growth and development of these trees. It was long known that the Rhizosphere activities of some plant stimulates beneficial microorganisms which helps in plant growth and plant disease management. The rhizosphere is the zone where the huge amount microorganism are present which also contains sloughed-off cells and chemicals released by roots provide ready food source for growth. Microbes can make nutrients and minerals in the soil available to plants, produce hormones that spur growth and stimulate plant immune system. Most of the soil borne pathogens are adopted to grow and survive in the bulk soil, but the rhizosphere is the playground and infection court where the pathogen establishes a parasitic relationship with the plant. Biocontrol has become an attractive alternative strategy for the management of plant diseases to reduce the excessive use of agrochemicals and its health hazards. There are various naturally occurring soil microbes that aggressively attack on plant pathogens and benefit plant by disease suppression (Singh, 2014) [23]. The collections of the fungal isolates from the rhizosphere of the medicinal plants basil (*Ocimum basilicum*), peppermint (*Mentha piperita*) and (*Aloe vera*) were assessed for *in vitro* antagonistic activity against phytopathogenic fungi *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Verticillium dahliae*.

Now a days the scientists are working in agriculture field are using the soil from the root zone of banyan tree for management of soil borne diseases in fruit crops. Therefore present studies were undertaken to study *in vitro* comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfsii* Sacc.

### Materials and Methods

Present studies were carried out in the laboratory of Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. The materials and methods adopted to complete the studies undertaken were described in this chapter under following appropriate heads.

### Experimental Materials

The various kinds of materials viz., bacteriocides, chemicals, glasswares, culture media and other miscellaneous items required for conducting present studies were obtained from the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

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### Laboratory facilities

Whole experiment was planned and conducted in the department laboratory, Department of plant pathology, College of Agriculture, Parbhani. *In vitro* studies/experiments were conducted in the laboratory of the Department of Plant Pathology, College of Agriculture, Parbhani.

### Methods

#### Sample Collection

Total 16 soil samples were collected from the rhizosphere of road side banyan tree which were deforested during the widening of road. The soil samples were taken from the depth of 15 cm i.e., rhizospheric area of banyan tree. The samples were collected and taken to the laboratory of Department of Plant Pathology, College of Agriculture, Vasant Rao Naik Marathwada Agricultural University, Parbhani. For isolation of different micro-organisms and their *in vitro* effects on some soil borne plant pathogens.

**Table:** Details of Location and Month of collection of soil samples for isolation of mycoflora

Sr. No.	District	Tehsil	Location	Number of Samples	Month / Year
1.	Parbhani	Parbhani	Gangakhed Road	1	March 2019
2.	Parbhani	Parbhani	Gangakhed Road	1	March 2019
3.	Parbhani	Gangakhed	Gangakhed Road	1	March 2019
4.	Parbhani	Parbhani	Pathri Road	1	March 2019
5.	Parbhani	Parbhani	Pathri Road	1	March 2019
6.	Parbhani	Pathri	Pathri Road	1	March 2019

#### Isolation of Micro-organisms

Serial dilution technique was used to isolate micro-organisms from the collected soil samples. These soil samples were air dried in shade and well grind before using for isolation by serial dilutions. Potato Dextrose Agar and Nutrient Agar medium used for isolation and growth. Isolation was carried out under aseptic conditions.

The test tubes then labelled as  $10^{-1}$ ,  $10^{-2}$  and  $10^{-9}$ . Each test tube was filled with 9ml of distilled water. These test tubes were plugged with non absorbent cotton and were sterilized in an autoclave as mentioned earlier. After cooling initial dilution was prepared in test tube labelled as  $10^{-1}$  with the addition of 1 gm soil into the first test tube containing 9 ml of distilled water. This test tube were rolled to and fro between the palms of hands for 5 to 10 minutes for mixing the content uniformly and also to obtain uniform distribution of the soil sample. From the first dilution 1ml of suspension was transferred to the test tube labelled as  $10^{-2}$  containing 9 ml of distilled water with the help of sterilized pipette.

The same procedure was repeated till the original sample was diluted to  $10^{-9}$ . Each time sterilized pipette were used. Then sterilized plates which were heated at  $180^{\circ}\text{C}$  for 2 hours Hot Air Oven. From these some plates were poured with PDA medium and some with NA medium for isolation of fungus and bacteria respectively. While pouring the media was sterilized in Autoclave at 15 lbs and cooled up to  $45^{\circ}\text{C}$ . Also added antibacterial agents in PDA. The media were poured about 15-20ml/plate. Then allowed plates to solidify. After that the 1ml suspension from dilution  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  for fungus isolation on PDA and from dilution  $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$  for isolation of bacteria NA (while rotating the test tubes in

between the palms of hands) were placed on solidified medium and spread it thoroughly on it. Plates were packed with tape and labelled with marker. Plates were incubated in inverted position in BOD incubator at  $\pm 28^{\circ}\text{C}$  up to 2 days for bacteria and up to 7 days for fungus. The plates were observed every day for growth of micro organisms and bits of their growth from growing colonies were transferred on sterilized PDA slants for fungus and NA slants for bacteria.

#### Purification of fungal cultures

Isolated cultures of fungi from the rhizosphere sample were transferred on to sterilized PDA media in petri plates under aseptic condition. After the Growth of fungi on plates bits of growing hypha of the fungus were transferred to the PDA slants in the test tube in aseptic condition.

#### Purification of Bacterial cultures

Isolated cultures of bacteria from rhizosphere sample were transferred on sterilized NA medium on petri plates with the help of inoculating needle having loop at the tip. After the growth of bacterial culture on plates the loop full of suspension from the plates were transferred on NA slants in test tubes.

#### Maintenance of the cultures

Isolated fungal and bacterial cultures were maintained on sterilized PDA and NA slants respectively in the refrigerator at about 8 to  $10^{\circ}\text{C}$ . Periodic transfer of the culture were carried out of sterilized PDA and NA slants to keep cultures in active growth.

#### Culture of the Pathogens

The cultures of the pathogens *Viz. Fusarium oxysporum* f. sp. *ciceri*, *Pythium aphanidermatum*, *Sclerotium rolfsii* were obtained from the Department of Plant Pathology, VNMKV Parbhani. Which were already identified and tested for pathogenicity.

#### Mass multiplication of Bio-control agents

Fungal cultures were multiplied on sterilized Potato Dextrose Agar medium in petri plates. For these 20 ml of medium had temperature  $45^{\circ}\text{C}$  were poured in each 90 mm petri plate. These plates were inoculated with a bit of growth of fungus. The fungal cultures were incubated in an incubator at  $28^{\circ}\pm 2^{\circ}\text{C}$  temperature for 7 days.

Bacterial cultures were multiplied on sterilized Nutrient Agar Medium in Petri plates for this 20ml of medium had temperature  $45^{\circ}\text{C}$  were poured in each petri plate. The plates were inoculated with growing bacterial cultures. These bacterial cultures were incubated in an incubator at  $28^{\circ}\pm 2^{\circ}\text{C}$  temperature for 2 days.

#### Bio-control agents available at VNMKV, Parbhani

The strains of biocontrol agents available at Department of Plant Pathology, VNMKV Parbhani were also assessed against soil borne pathogens *viz. Fusarium oxysporum* f. sp. *ciceri*, *Pythium aphanidermatum* and *Sclerotium rolfsii* by Dual Culture Technique on PDA medium. The isolated biocontrol agents were assessed against soil born pathogen *Pythium aphanidermatum* by Dual Culture Technique on PDA medium as per procedure described by Dennis and Webster (1971). For this procedure 20 ml of PDA media ( $45^{\circ}\text{C}$ ) were poured in each 90 mm diameter Petri plates allowed

to solidify. A 5 mm disc (with the help of cork borer) of *Pythium aphanidermatum* as per the need were placed on one end of the medium with the help of sterilized needle. Just opposite to it 5 mm disc of needed biocontrol agent were placed. Control plate also maintained. Then the plates will be incubated at 28±2 °C. On seventh day after incubation, the growth of pathogenic test organisms will be measured and percent growth inhibition was calculated using the formula (Ranjana Chakrabarty *et al.*, 2013) [3].

$$\text{Percent Growth inhibition (I)} = \frac{C - T}{C} \times 100$$

Where

I= Percent inhibition over control

C= Radial growth in control

T= Radial growth in treatment

### The experimental details

Design: CRD

Replications: 3

Treatments: 9

### Results and Discussion

In the present investigations the soil samples for the isolation of microorganisms were collected from the rhizosphere of road sides Banyan trees which were deforested during the widening of road. The soil samples were taken from the location of Gangakhed and Pathri roads of Parbhani District.

#### Isolation of fungi from rhizosphere of Banyan trees

The fungal species from the rhizospheric soils was isolated by using 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> dilutions by serial dilution technique. The results of isolations are presented in Table-2. On the basis of morphology and colony characters, the fungal species were identified. Six samples were collected from the different Banyan trees. The samples were collected from the location of Gangakhed and Pathri road of Parbhani district. The microorganisms from these samples were isolated on PDA medium by using serial dilution method. These rhizospheric soil samples contained the population of *Trichoderma* spp. and *Aspergillus niger* which were identified on the basis of morphology and colony characters.

The results presented in Table 1. Revealed that, *Trichoderma* spp. were mostly present in collected soil samples. *Trichoderma harzianum* was isolated from the soil sample which was collected from Gangakhed road of Parbhani District. *Trichoderma hamatum* was isolated from another soil sample collected from the location of Gangakhed road from Parbhani Tehsil of Parbhani District. *Trichoderma asperellum* and *Trichoderma koningi* was isolated from the soil sample which was collected from the location of Gangakhed road which comes under Gangakhed Tahsil of Parbhani District. *Trichoderma longibrachiatum* and *Aspergillus niger* was isolated from the soil sample collected from the location of Pathri Road which comes under Parbhani Tehsil of Parbhani District.

#### Isolation of bacteria from the rhizosphere of Banyan trees

*Bacillus* and *Pseudomonas* species were isolated from the rhizospheric collected from the rhizosphere of Banyan tree located at Gangakhed and Pathri Road of district Parbhani.

*Bacillus subtilis* was isolated from the rhizospheric soil

samples collected from the location of Gangakhed road which comes under Parbhani Tehsil of Parbhani District. *Pseudomonas fluorescens* was isolated from soil sample collected from the location of Pathri road which comes under Parbhani Tehsil of Parbhani district. *Pseudomonas striata* was isolated from the soil sample collected from the location of Pathri road which comes under Pathri Tehsil of Parbhani District.

The same type of results for isolation of fungal and bacterial spp. from rhizosphere soil were found by workers *viz.* Kannan *et al.*, (2009) [9]; Panaiyadian and Chellaia (2011) [15]; Ahmed *et al.* (2014) [11]; Srinivas *et al.* (2015) [25]; Azizpour and Rouhrazi (2016) [2]; Damam *et al.* (2016) [4] and Rao *et al.* (2016) [21].

**Table 1:** Collection of soil sample from rhizosphere of Banyan trees

Sr. No.	District	Tehsil	Location	Sample Code
1.	Parbhani	Parbhani	Gangakhed Road	PBN 1
2.	Parbhani	Parbhani	Gangakhed Road	PBN 2
3.	Parbhani	Gangakhed	Gangakhed Road	GGK 3
4.	Parbhani	Parbhani	Pathri Road	PBN 4
5.	Parbhani	Parbhani	Pathri Road	PBN 5
6.	Parbhani	Pathri	Pathri Road	PTR 6

#### Comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfsii*.

The comparison of isolated rhizospheric biocontrol agents were done with the available biocontrol agents at Department of Plant pathology, VNMKV Parbhani against *Sclerotium rolfsii*. The data so obtained is presented in Table-1 Fig-1 and Plates-I and II. The data revealed that the *Trichoderma harzianum* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 33 mm with 63.33 per cent inhibition, whereas, in the treatment of VNMKV *Trichoderma harzianum*, the test pathogen showed 31 mm mycelial growth with 65.55 per cent inhibition.

The *Trichoderma hamatum* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 31.33 mm with 65.18 per cent inhibition, whereas, in the treatment of VNMKV *Trichoderma hamatum*, the test pathogen showed 31mm mycelial growth with 65.55per cent inhibition.

The *Trichoderma asperellum* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 31.33 mm with 65.18 per cent inhibition, whereas, in the treatment of VNMKV *Trichoderma asperellum*, the test pathogen showed 30 mm mycelial growth with 66.66 per cent inhibition.

The *Trichoderma koningii* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 28.33 mm with 68.51 per cent inhibition, whereas, in the treatment of VNMKV *Trichoderma koningi*, the test pathogen showed 28.33 mm mycelial growth with 68.51 per cent inhibition. The *Trichoderma longibrachiatum* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 28 mm with 68.88 per cent inhibition, whereas, in the treatment of VNMKV *Trichoderma longibrachiatum*, the test pathogen showed 26.66 mm mycelial growth with 70.36 per cent inhibition.

The *Aspergillus niger* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 31 mm with 65.55 per cent inhibition, whereas, in the treatment of



VNMKV *Aspergillus niger*, the test pathogen showed 28 mm mycelial growth with 68.88 per cent inhibition.

The *Pseudomonas fluorescens* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 24 mm with 73.25 per cent inhibition, whereas, in the treatment of VNMKV *Pseudomonas fluorescens*, the test pathogen showed 27.33 mm mycelial growth with 69.62 per cent inhibition.

The *Pseudomonas striata* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 23 mm with 74.44 per cent inhibition, whereas, in the treatment of VNMKV *Pseudomonas striata*, the test pathogen showed 25.33 mm mycelial growth with 72.84 per cent inhibition.

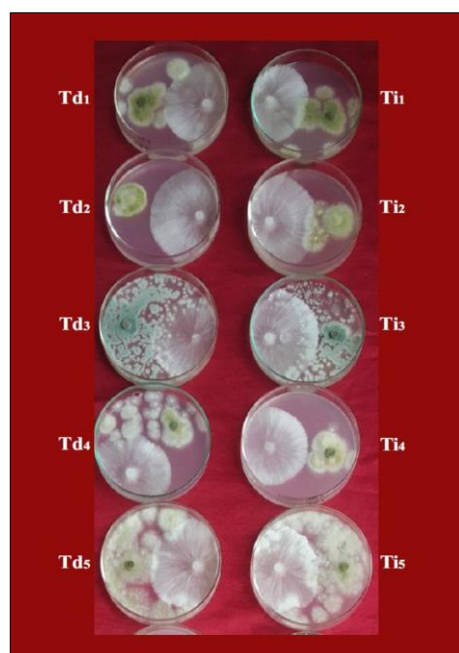
The *Bacillus subtilis* isolated from rhizosphere of banyan trees showed the mycelial growth of test pathogen as 24.66

mm with 73.92 per cent inhibition, whereas, in the treatment of VNMKV *Bacillus subtilis*, the test pathogen showed 25mm mycelial growth with 72.21 per cent inhibition.

From all above observations it is revealed that isolated strains of isolated *Pseudomonas fluorescens*, *Pseudomonas striata* and *Bacillus subtilis* were more antagonists as it showed more inhibition i.e. 73.25%, 74.44% and 73.92% respectively of test pathogen as against the VNMKV strains of *fluorescens*, *Pseudomonas striata* and *Bacillus subtilis* which showed inhibition 69.62%, 72.84% and 72.21% respectively of test pathogen. The isolated strains of *Trichoderma hamatum* (65.18%), *Trichoderma asperellum* (65.18%), and *Trichoderma koningi* (68.51%) were at par to the VNMKV *Trichoderma hamatum* (65.65%), *Trichoderma viride* (66.66%) and *Trichoderma koningi* (68.51%), respectively.

**Table 2:** Comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfsii* Sacc.

Tr. No.	Treatments	Isolated biocontrol agents at 5 <sup>th</sup> DAI		Available biocontrol agents at 5 <sup>th</sup> DAI	
		Col. Dia.* (mm)	% Inhibition*	Col. Dia.* (mm)	% Inhibition*
T1	<i>Trichoderma harzianum</i>	33.000	63.330 (52.73)	31.000	65.550 (54.05)
T2	<i>Trichoderma hamatum</i>	31.333	65.180 (53.83)	31.000	65.550 (54.05)
T3	<i>Trichoderma asperellum</i>	31.333	65.180 (53.83)	30.000	66.660 (54.73)
T4	<i>Trichoderma koningii</i>	28.333	68.513 (55.86)	28.333	68.513 (55.86)
T5	<i>Trichoderma longibrachiatum</i>	28.000	68.883 (56.09)	26.667	70.367 (57.01)
T6	<i>Aspergillus niger</i>	31.000	65.550 (54.05)	28.000	68.883 (56.09)
T7	<i>Pseudomonas fluorescens</i>	24.667	73.253 (58.85)	27.333	69.627 (56.55)
T8	<i>Pseudomonas striata</i>	23.000	74.440 (59.63)	25.333	72.847 (58.59)
T9	<i>Bacillus subtilis</i>	24.667	73.920 (59.29)	25.000	72.217 (58.19)
T10	Control	90	0 (0)	90	0 (0)
	S.E. ±	1.135	1.036	1.308	1.500
	C.D. (P=0.01)	3.373	3.077	3.886	4.458



Td<sub>1</sub>: *Trichoderma harzianum*      Ti<sub>1</sub>: *Trichoderma harzianum*  
 Td<sub>2</sub>: *Trichoderma hamatum*      Ti<sub>2</sub>: *Trichoderma hamatum*  
 Td<sub>3</sub>: *Trichoderma viride*      Ti<sub>3</sub>: *Trichoderma viride*  
 Td<sub>4</sub>: *Trichoderma koningi*      Ti<sub>4</sub>: *Trichoderma koningi*  
 Td<sub>5</sub>: *Trichoderma longibrachiatum*      Ti<sub>5</sub>: *Trichoderma longibrachiatum*

**Note:** Td: Treatment with biocontrol agents of VNMKV, Parbhani.  
**Ti:** Treatment with isolated rhizospheric microflora

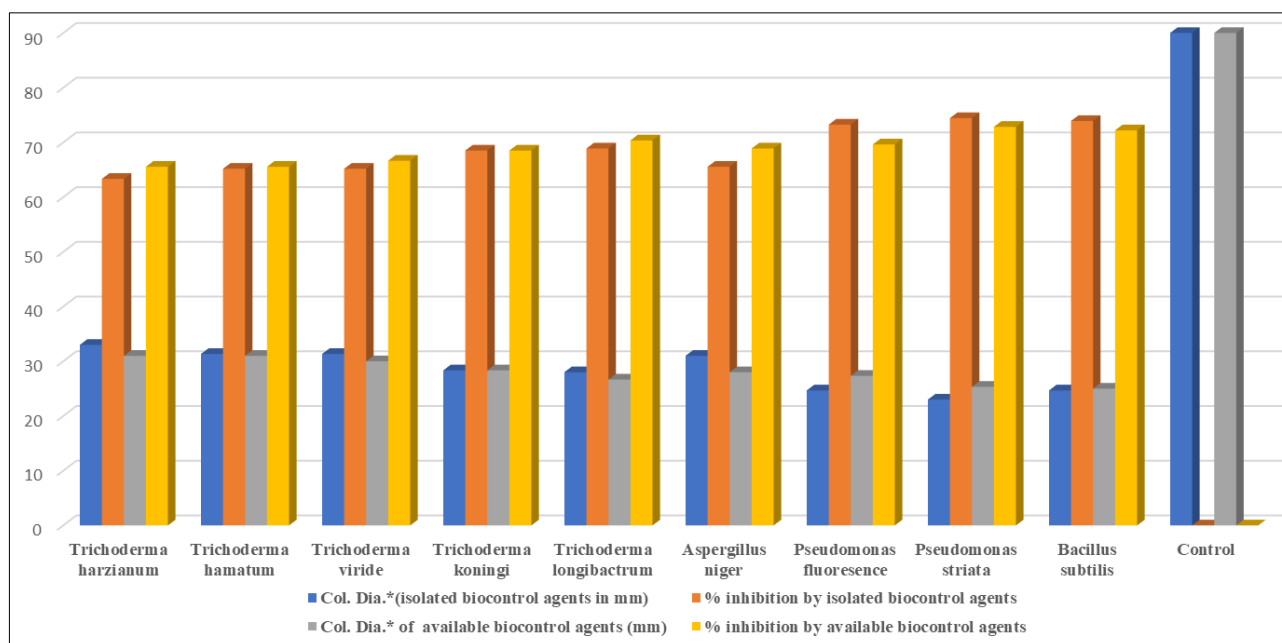


Td<sub>6</sub>: *Aspergillus niger*      Ti<sub>6</sub>: *Aspergillus niger*  
 Td<sub>7</sub>: *Pseudomonas fluorescens*      Ti<sub>7</sub>: *Pseudomonas fluorescens*  
 Td<sub>8</sub>: *Pseudomonas striata*      Ti<sub>8</sub>: *Pseudomonas striata*  
 Td<sub>9</sub>: *Bacillus subtilis*      Ti<sub>9</sub>: *Bacillus subtilis*  
 Td<sub>10</sub>: Control      Ti<sub>10</sub>: Control

**Note:** Td: Treatment with biocontrol agents of VNMKV, Parbhani.  
**Ti:** Treatment with isolated rhizospheric microflora

**Plate 1:** Comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfsii* Sacc.

**Plate 2:** Comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfsii* Sacc.



**Fig 1:** Comparison of isolated rhizospheric microflora with biocontrol agents of VNMKV, Parbhani against *Sclerotium rolfisii* Sacc.

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