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11. Isolation and Screening of Bacillus spp. for Microbiological Control of Sclerotium Rolfsii Sacc., A Stem Rot Pathogen of Groundnut

R. R. Rakh
Department of Microbiology, Sant Tukaram College of Arts and Science, Parbhani.
L. S. Raut
Department of Microbiology, Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.).
S. M. Dalvi
Department of Botany, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.).

Abstract

Stem rot is one of the most important disease of groundnut caused by *Sclerotium rolfsii* Sacc. which causes major crop losses. The present study was undertaken to search for the effective *Bacillus spp.* for microbiological control of *Sclerotium rolfsii* Sacc. 129 *Bacillus spp.* were isolated from different rhizospheric niches of healthy plants, and screened *in vitro* against *Sclerotium rolfsii*, by dual culture technique. Out of these *Bacillus spp.*, *Bacillus spp. 57* found effective in managing the phytopathogen by dual culture technique.

Key words: Groundnut, Stem rot, *Sclerotium rolfsii*, *Bacillus spp.*

1.0 Introduction

Stem rot caused by *Sclerotium rolfsii*, a broad host range fungus, is the major soil borne disease of groundnut (*Arachis hypogaea*). In India among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. Stem-rot caused by *S. rolfsii* is sporadic in most of the groundnut growing areas like Tamil Nadu, Andhra Pradesh, Karnataka (Pande, *et al.*, 2000).

The traditional agricultural practice to control the phytopathogen *S. rolfsii* is by using variety of fungicides e.g. Bavistin, Captan etc. but a severe disadvantage of the traditional method is that it is not effective to check the *Sclerotium* during the cropping period (90- 100 days) and is not eco-friendly. Because of the increased usage of chemical fungicides produced concern for the environment and human health, microbial inoculants have been experimented

extensively during the last decade to control wilt and other plant diseases (Siddiqui and Shakeel, 2006; Chakraborty and Chatterjee, 2008; Akhtar *et al.*, 2010).

Bacteria have been explored as microbiological control agents for plant diseases (Gerhardson, 2002) and as plant growth promoters and inducers of disease resistance (Catellan *et al.*, 1999; Bargabus *et al.*, 2002; Bais *et al.*, 2004). Apart from improving plant health, they also meet the increasing demand for low-input agriculture.

The use of antagonistic bacteria is reported as a powerful strategy to suppress soil-borne pathogens due to their ability to antagonize the pathogen by multiple modes and to effectively colonize the rhizosphere. The widely known mechanisms of biocontrol action are competition for an ecological niche or substrate, as well as the production of inhibitory compounds and hydrolytic enzymes that are often active against a broad spectrum of fungal pathogens. Many microorganisms are known to produce multiple antibiotics which can suppress one or more pathogens (Haas and Defago, 2005; Stein, 2005; Ge *et al.*, 2007). For instance, *Bacillus subtilis* produces several ribosomal and non-ribosomal peptides that act as antibiotics such as iturins, surfactins and zwittermycin (Asaka and Shoda, 1996; Stein, 2005) and it secretes also hydrolytic enzymes, i.e. protease, glucanase (Cazorla *et al.*, 2007), chitinase (Manjula *et al.*, 2004), lipase (Detry *et al.*, 2006) and amylase (Konsoula and Liakopoulou-Kyriakides, 2006).

Pseudomonas spp. and *Bacillus* spp. have been applied as biocontrol agents to suppress plant-pathogenic organisms (Koumoutsi *et al.*, 2007; Joseph *et al.*, 2008; Akhtar *et al.*, 2010). In particular *Bacillus* spp. is gaining recognition as safe biocontrol agents in a variety of crops, specifically as seed protectants and antifungal agents (Asaka and Shoda, 1996; Stein, 2005). Moreover, they are spore-formers, which impart a natural formulation advantage over other microorganisms (Emmert and Handelsman, 1999; Haas and Defago, 2005; Romero *et al.*, 2007).

The objective of the current study was to isolate particularly *Bacillus spp.*, from rhizospheric niches of healthy plants such as Neem and evaluate its potential *in vitro* in controlling the soil-borne pathogen, *Sclerotium rolfsii*, causing stem rot of groundnut by dual culture method.

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2.0 Materials and Methods

2.1 Stem rot pathogen of Groundnut

Sclerotium rolfsii, Stem rot pathogen of groundnut had been isolated in our laboratory in previous studies (Rakh, 2010). Fungal culture of *Sclerotium rolfsii* was maintained on potato dextrose agar (PDA) by sub-culturing at regular intervals.

2.2 Isolation of *Bacillus spp.* from Rhizospheric niches

Soil from rhizospheric niches of different healthy plants such as neem, soybean, tur etc. were collected in poly-ethylene bags and brought to the research laboratory. 1 gm of soil sample was inoculated into 100 ml nutrient broth and kept for incubation at room temperature for 24 h. For isolation of *Bacillus* isolate, a modified method of Kim *et al.*, (1997) was employed. A 1ml of enriched Nutrient Broth was added to 10 ml sterile distilled water and kept at 80°C for 20 min. later on a loopful of culture was streaked on nutrient agar plates. Plates were incubated at room temperature for 48 hr. Typical white colonies were picked up individually and purified on nutrient agar slants. All the isolates were tentatively named during this research to avoid confusion.

2.3 Screening for Potential Microbiological Control agents

All the *Bacillus spp.* were screened for potential antagonistic activity against *S. rolfsii*, on King's B agar (Ran, *et al.*, 2003) using dual culture technique. (Rangeshwaran and Prasad, 2000) An agar disc (5 mm) was cut from an actively growing (96 h) *S. rolfsii*, and placed on the surface of fresh King's B Agar medium at the one side of the Petri plates. A loopful of actively growing *Bacillus spp.* (each) was placed opposite to the fungal disc. Plates inoculated with phytopathogen and without bacteria were used as control. Each experiment was carried out in triplicates. Plates were incubated at room temperature for 7 days. Degree of antagonism was determined by measuring the radial growth of pathogen with bacterial culture and control and Percent inhibition was calculated by the following equation (Riungu *et al.*, 2008).

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter of pathogen alone (Control)} - \text{Colony diameter of pathogen - Antagonist}}{\text{Colony diameter of pathogen alone}} \times 100$$

3.0 Result and Discussion

3.1 Isolation of Rhizobacteria

In present work, 129 *Bacillus spp.* were isolated from rhizospheric niches of different healthy plants such as Soybean, Neem, Tur etc. All the rhizospheric *Bacillus spp.* were tentatively named as *Bacillus spp* 1 to *Bacillus spp* 129.

3.2 *In Vitro* Screening for Microbiological Control agents against *Sclerotium rolfsii*:

To search for potential microbiological control agent, the entire 129 *Bacillus spp.* screened for their antagonistic activity against *S. rolfsii*, by dual culture method. The present study has shown that *Sclerotium rolfsii*, the stem rot pathogen of groundnut can be controlled by *Bacillus spp 57* recovered from the rhizospheric niche. *Bacillus spp 57* was found effective in inhibiting the phytopathogen, *Sclerotium rolfsii in vitro* (56.66 %) in contrast to other *Bacillus spp.* isolated from various source (Photo Plate 1 & Table 1).

This result was in correlation with the result obtained by Chen *et al.* (2004). Similar findings were also recorded by the study conducted by Souto *et al.* (2004) where mycelial growth of *Sclerotium spp.* was inhibited by application of *Bacillus spp.* using the dual culture technique. Similarly findings were also shown by *Bacillus subtilis* which reduced the growth of *S. rolfsii* effectively on PDA when compared with the control (Keyser and Ferreira, 1988) & also by Gomashe *et al.*, (2014) where *Bacillus subtilis* found effective in controlling *Sclerotium rolfsii* by producing bioactive compound.

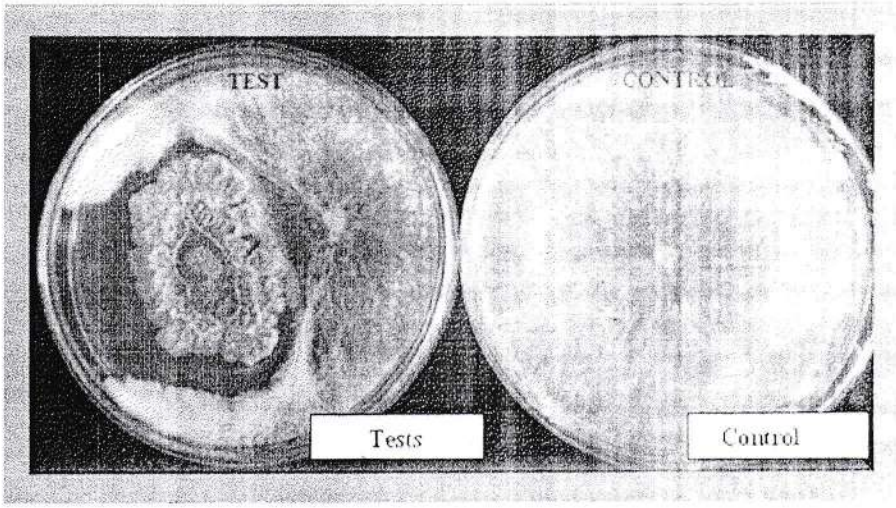

Photo Plate 2: *In Vitro* Antagonism of *Bacillus spp.* 57 **against** *Sclerotium rolfsii* **by Dual Culture Technique**

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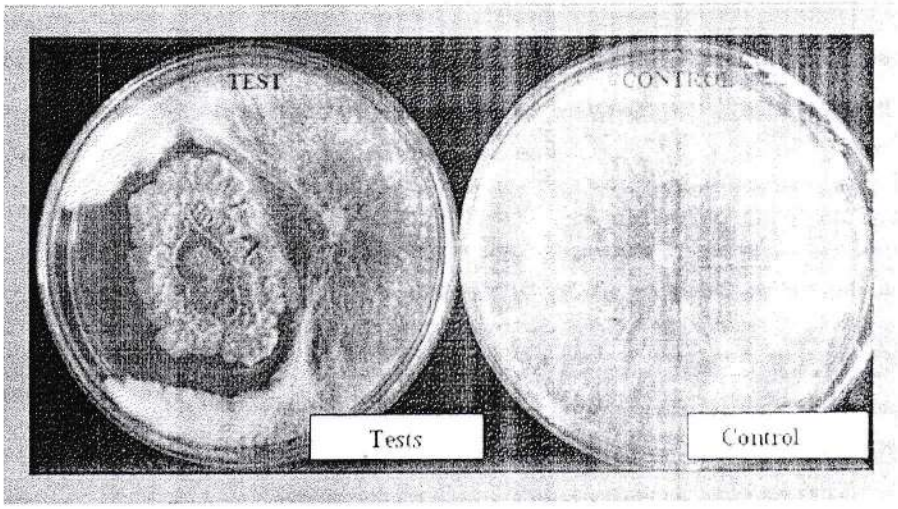

Photo Plate 2: *In Vitro* Antagonism of *Bacillus spp.* 57 against *Sclerotium rolfsii* by Dual Culture Technique

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Tentative Name of Bacteria	Inhibition of *S. rolfsii* (%)	Tentative Name of Bacteria	Inhibition of *S. rolfsii* (%)	Tentative Name of Bacteria	Inhibition of *S. rolfsii* (%)
Bacillus spp. 1	21.2	*Bacillus spp. 44*	29.4	*Bacillus spp. 87*	42.6
Bacillus spp. 2	35.3	*Bacillus spp. 45*	21.8	*Bacillus spp. 88*	24.2
Bacillus spp. 3	43.1	*Bacillus spp. 46*	10.9	*Bacillus spp. 89*	33.1
Bacillus spp. 4	12.1	*Bacillus spp. 47*	16.1	*Bacillus spp. 90*	43.2
Bacillus spp. 5	21.0	*Bacillus spp. 48*	20.5	*Bacillus spp. 91*	41.2
Bacillus spp. 6	30.4	*Bacillus spp. 49*	21.5	*Bacillus spp. 92*	36.5
Bacillus spp. 7	19.0	*Bacillus spp. 50*	32.1	*Bacillus spp. 93*	39.2
Bacillus spp. 8	12.5	*Bacillus spp. 51*	23.1	*Bacillus spp. 94*	14.2
Bacillus spp. 9	21.4	*Bacillus spp. 52*	12.0	*Bacillus spp. 95*	23.6
Bacillus spp. 10	16.9	*Bacillus spp. 53*	19.8	*Bacillus spp. 96*	45.8
Bacillus spp. 11	31.7	*Bacillus spp. 54*	13.1	*Bacillus spp. 97*	34.7
Bacillus spp. 12	33.0	*Bacillus spp. 55*	25.8	*Bacillus spp. 98*	11.5
Bacillus spp. 13	27.0	*Bacillus spp. 56*	8.7	*Bacillus spp. 99*	5.3
Bacillus spp. 14	36.4	***Bacillus spp. 57***	**56.6**	*Bacillus spp. 100*	3.0
Bacillus spp. 15	20.2	*Bacillus spp. 58*	5.8	*Bacillus spp. 101*	0.0
Bacillus spp. 16	16.0	*Bacillus spp. 59*	13.2	*Bacillus spp. 102*	37.5
Bacillus spp. 17	11.0	*Bacillus spp. 60*	28.1	*Bacillus spp. 103*	0.0
Bacillus spp. 18	14.0	*Bacillus spp. 61*	33.7	*Bacillus spp. 104*	16.8
Bacillus spp. 19	23.0	*Bacillus spp. 62*	10.3	*Bacillus spp. 105*	0.0
Bacillus spp. 20	26.9	*Bacillus spp. 63*	35.2	*Bacillus spp. 106*	35.2
Bacillus spp. 21	25.0	*Bacillus spp. 64*	41.7	*Bacillus spp. 107*	18.6
Bacillus spp. 22	18.7	*Bacillus spp. 65*	31.6	*Bacillus spp. 108*	41.5
Bacillus spp. 23	13.4	*Bacillus spp. 66*	46.8	*Bacillus spp. 109*	4.2
Bacillus spp. 24	15.7	*Bacillus spp. 67*	32.8	*Bacillus spp. 110*	0.0
Bacillus spp. 25	25.0	*Bacillus spp. 68*	15.6	*Bacillus spp. 111*	47.9
Bacillus spp. 26	13.8	*Bacillus spp. 69*	14.6	*Bacillus spp. 112*	37.8
Bacillus spp. 27	26.5	*Bacillus spp. 70*	27.3	*Bacillus spp. 113*	0.0
Bacillus spp. 28	34.2	*Bacillus spp. 71*	45.8	*Bacillus spp. 114*	17.4
Bacillus spp. 29	11.8	*Bacillus spp. 72*	41.5	*Bacillus spp. 115*	19.3
Bacillus spp. 30	14.3	*Bacillus spp. 73*	37.4	*Bacillus spp. 116*	28.4
Bacillus spp. 31	31.1	*Bacillus spp. 74*	48.2	*Bacillus spp. 117*	27.6
Bacillus spp. 32	25.1	*Bacillus spp. 75*	26.4	*Bacillus spp. 118*	0.0
Bacillus spp. 33	21.9	*Bacillus spp. 76*	35.8	*Bacillus spp. 119*	19.7
Bacillus spp. 34	23.2	*Bacillus spp. 77*	11.5	*Bacillus spp. 120*	43.7
Bacillus spp. 35	38.3	*Bacillus spp. 78*	23.4	*Bacillus spp. 121*	35.9
Bacillus spp. 36	27.4	*Bacillus spp. 79*	14.7	*Bacillus spp. 122*	31.0

Bacillus spp. 37	11.3	*Bacillus spp. 80*	21.7	*Bacillus spp. 123*	29.7
Bacillus spp. 38	24.8	*Bacillus spp. 81*	29.0	*Bacillus spp. 124*	0.0
Bacillus spp. 39	9.8	*Bacillus spp. 82*	15.2	*Bacillus spp. 125*	17.4
Bacillus spp. 40	17.3	*Bacillus spp. 83*	19.4	*Bacillus spp. 126*	14.9
Bacillus spp. 41	23.1	*Bacillus spp. 84*	23.9	*Bacillus spp. 127*	0.0
Bacillus spp. 42	25.3	*Bacillus spp. 85*	31.4	*Bacillus spp. 128*	38.9
Bacillus spp. 43	13.1	*Bacillus spp. 86*	46.3	*Bacillus spp. 129*	35.7

Table1: *In Vitro* Screening of *Bacillus spp.* against *Sclerotium rolfsii* by dual culture technique

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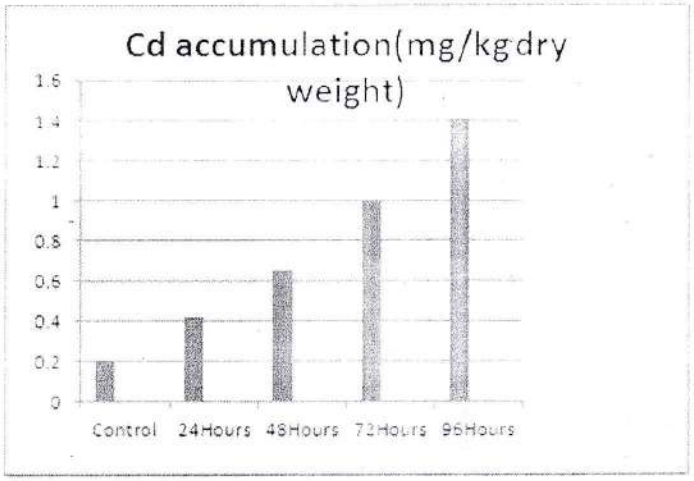
TABLE: Cadmium content(mg/kg dry weight) in soft tissues of *Corbicula striatella* after acute treatment.



The flow of elements through different trophic level through food chain. Fish are feed on algae, zooplanktons and aquatic plants. Fish are the major bottom feeders in the ecosystem which also have tremendous capacity to accumulate all the microelements present in their food. Fish are considered as the main bioaccumulators of pesticides, heavy metals, toxic chemicals etc. Heavy metals are the class of highly toxic elements, causing great health problem to human life through bioaccumulation from the fish.

Copper bioaccumulation and depuration by red. swamp crayfish, procambarus clarkia was observed by Nagvi et-al. (1998). They concluded that crayfish has a great potential for rapid accumulation and depuration of Cu in freshwater metal concentration in tissues of the freshwater fish, *Capoeta barroisi* from the sehan river was reported by Kargin (1998). Fung et-al. (2004) reported that due to industrial activity the heavy metal concentration such as, As, Cd, Cr, Ni, Pb, Se, Zn, Fe, and Hg, were increased in the body of *Perna viridis* and *Mytilus edulis* in the east coast of china.

Conclusion

The results obtained in this investigation showed great correlation with these obtained by other workers in this field. In response to increased concentrations of cadmium in *Corbicula striatella* accumulates high levels of cadmium.

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