PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Role of Rhizobia in Suppressing the Root Diseases of Soybean Under Soil Amendment

O Papel dos Rizóbios na Supressão de Doenças Radiculares da Soja sob Correção do Solo

PARVEEN, G.[1]
NOREEN, R.[1]
SHAFIQUE, H.A.[1]
SULTANA, V.[1]
EHTESHAMUL-HAQUE, S.[1]
ATHAR, M.[1, 2,*]

ABSTRACT - Rhizobia are soil bacteria, characterized by their unique ability to colonize the roots of leguminous crops, where they form nitrogen fixing nodules. Considerable evidence has been accumulated to identify the benefits associated with use of rhizobia as biocontrol agents against soil-borne pathogens, in addition to biological nitrogen fixation. In this study, out of four rhizobial isolates tested, *Bradyrhizobium* sp. inhibited the radial growth of all the test fungi viz: *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum*, while other 3 rhizobial isolates inhibited the growth of at least three fungi. Cell free culture filtrates of rhizobia also showed nematicidal activity by killing second-stage juveniles of *Meloidogyne javanica* at varying degrees. Application of rhizobial isolates alone or in mustard cake amended soil significantly ($p<0.05$) by suppression of root rotting fungi and root knot nematode on soybean roots. Rhizobia-treated plants showed less penetration of nematodes in roots than untreated control plants. Efficacy of rhizobia was found to increase against nematodes in mustard cake at 1% amended soil. Rhizobia also improved soybean growth by producing taller plants although plants showed poor nodulation. The tallest plant was found in some nitrogen fixing bacteria (NFB) + mustard cake (1%) treatment, but in most cases, the combined application did not offer any added advantage.

Keywords: mustard cake, rhizobia, soybeans.

RESUMO- Os rizóbios são bactérias que vivem no solo, caracterizadas pela capacidade única de colonizar as raízes das culturas de leguminosas, onde formam nódulos de fixação de nitrogênio. Foram acumuladas diversas evidências com o objetivo de identificar os benefícios associados ao uso de rizóbios como agentes de controle biológico contra patógenos de solo, além da fixação biológica de nitrogênio. Neste estudo, dos quatro isolados de rizóbios testados, ***Bradyrhizobium*** *sp. inibiu o crescimento radial de todos os fungos de teste, a saber:* ***Macrophomina phaseolina***, ***Rhizoctonia solani***, ***Fusarium solani*** *e* ***F. oxysporum***, *enquanto outros três isolados de rizóbios inibiram o crescimento de pelo menos três fungos. Os filtrados de cultura livre de células de rizóbios também apresentaram atividade nematicida, matando juvenis de segundo estágio de* ***Meloidogyne javanica*** *em graus variados. Os isolados de rizóbios aplicados sozinhos ou combinados com solo corrigido com torta de mostarda causaram redução significativa ($p<0,05$) da quantidade de fungos causadores da podridão de raízes, bem como de nematoides de galha, nas raízes da soja. As plantas tratadas com rizóbios apresentaram menor penetração de nematoides nas raízes do que as plantas de controle não tratadas. Foi observado aumento da eficácia dos rizóbios contra os nematoides no solo corrigido com bolo de mostarda a 1%. Os rizóbios também melhoraram o*

*** Corresponding author:**
<athar.tariq@cdfa.ca.gov>

Received: November 21, 2016
Approved: December 28, 2016

Planta Daninha 2019; v37:e019172336

[1] University of Karachi, Karachi-75270, Pakistan; [2] California Department of Food and Agriculture, Sacramento, CA 95832, USA.

Doi: 10.1590/S0100-83582019370100038

crescimento da soja, produzindo plantas mais altas, embora com nodulação deficiente. A planta mais alta foi encontrada no tratamento com torta de mostarda NFB-1 (1%), mas na maioria dos casos a aplicação combinada não proporcionou nenhuma vantagem adicional.

Palavras-chave: bolo de mostarda, rizóbios, soja.

INTRODUCTION

Bacteria belonging to the group of rhizobia are of considerable scientific and economic interest because of their ability to fix atmospheric nitrogen in leguminous plants (Brockwell et al., 1995; Spaink, 2000; Vasileva and Ilieva, 2012). Rhizobia induce the formation of root nodules in leguminous plants by the production of speciûc signal molecules called Nod factors (Spaink et al,. 1991; Sprent, 2001). Inside the nodules, rhizobia convert nitrogen into ammonia for uptake by host plants, which reduces the need for application of chemical fertilizers (Neeraj et al., 2009).

Moreover, along with nitrogen fixation efficiency of rhizobia, it also have a good potential of use as biological control agents against soilborne plant pathogens (Noreen et al., 2016). Some of the *Rhizobium* strains are known to reduce disease severity caused by *Pythium ultimum* (Ozkoc and Deliveli, 2001), *Phytophthora clandestine* (Simpfendorfer et al., 1999), *Fusarium solani* (Al Ani et al., 2012), *Fusariuum oxysporum*, *Rhizoctonia bataticola* and *Pythium* sp., (Nautiyal, 1997). Ehteshamul-Haque and Ghaffar (1993) found, under field conditions, that *Sinorhizobium meliloti*, *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* used either as seed dressing or as soil drench reduced infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., in both leguminous and non-leguminous plants.

Organic amendments have a positive effect on crop production and plant health and some of these effects have been related to the enhancement of soil suppressiveness against soil-borne pathogens (Bailey and Lazarovits, 2003). There are reports that application of botanical toxicants or plant products has reduced root knot disease (Aziz and Al-Askar, 2012; Khalil et al., 2012; Meyer et al., 2015) and root-rotting fungi (Shafique et al., 2015). Organic amendment of soil has also been reported to enhance the activity of biocontrol agents in the suppression of plant pathogens (Garbeva et al., 2004). Among the various oil cakes, mustard oil cake is inexpensive and easy to break down when applied to the soil, and it works as a conditioner. In addition to reducing viruses and nematodes, mustard oil cake increases plant survival rate and yields (Rafi et al., 2016).

Soybean (*Glycine max* L. Merill.) is one of the most important legume crops in the world and a major component of the diet of food-producing animals and humans (Friedman and Brandon, 2001). Soybean plants are subject to infection by several soil-borne pathogens that inducing root rot disease, which is considered one of the most important limiting factors against plant growth and yield (Yang and Feng, 2001). *Fusarium solani* and *Macrophomina phaseolina* are among the most important pathogens affecting soybean (Al-Ani et al., 2012). Suppression of root diseases of soybean with rhizobia or oil cakes have been reported. Thus, the present study describes the effect of soil amendment with mustard cake on the efficacy of rhizobia in reducing infections against soybean caused by root-infecting fungi and root knot nematodes.

MATERIALS AND METHODS

Microbial cultures: Cultures of *Bradyrhizobium* sp. (NFB-1), *Rhizobium* sp. (NFB-2), *Sinorhizobium meliloti* (NFB-28), *S. meliloti* (NFB-29) and *Trichoderma harzianum*, used in this study were collected from Karachi University Culture Collection (KUCC), Karachi.

In vitro antifungal activity of rhizobia

The effect of rhizobial strains on the growth of four root-infecting fungi viz., *Fusarium solani*, *F. xysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* was examined *in vitro*. The strains/isolates of rhizobia were streaked on one side of a Petri dish containing Czapek's Dox Agar, pH

7.2. On the other side of the Petri dish, 5 mm diameter discs of root infecting test fungi were inoculated (Ji et al., 2014). The dishes were incubated at 28 °C and radial growth of test organisms were measured daily.

Cell free culture filtrate of rhizobia

Bacterial isolates were grown in Yeast Extract Mannitol Broth at 30 °C for 48 h in the dark and centrifuged twice at 3000 rpm for 20 minutes. The pellets were discarded and culture filtrates were collected in beakers for use.

***In vitro* antifungal activity of cell free culture filtrate of rhizobia**

Thick sterilized filter paper discs were loaded with each filtrate at 20 µL/disc and dried. The loaded discs were placed at one side of the Petri dishes containing Czapek's Dox agar (pH 7.2). A 5 mm disc of actively growing culture of plant pathogens *Fusarium solani*, *F. oxysporum*, *R. solani* and *M. phaseolina* were inoculated on another side of the dish. There were three replicates of each filtrate and Petri dishes were incubated at 28 °C for 7 days. The distance between the fungal colony and the disc was considered as a zone of inhibition and measured in mm and averaged.

***In vitro* nematicidal activity of rhizobia**

One milliliter of freshly hatched second stage juvenile suspension (25-40 juveniles) and 1 mL cell free culture filtrate of bacterial isolates were transferred to glass cavity blocks and kept at 26±5 °C. There were three replicates of each treatment and juvenile mortality was recorded after 48 h.

Screen house experiment

Powdered mustard cake was mixed in sandy loam soil (300 g per pot), pH 8.0 at 1% and 3% w/w and transferred to 8 cm diameter plastic pots which were watered daily and kept at 50% water holding capacity (Keen and Raczkowski, 1921). After two weeks of soil amendment, aqueous suspension of rhizobial isolates NFB-1 (*Bradyrhizobium* sp.) (5.2 x 10^8 cfu mL^{-1}), NFB-2 (*Rhizobium* sp.) (5.0 x 10^8 cfu mL^{-1}), NFB-28 (*S. meliloti*) (6.3 x 10^8 cfu mL^{-1}), NFB-29 (*S. meliloti*) (5.0 x 10^8 cfu mL^{-1}), *T. harzianum* (7.0 x 10^8 cfu mL^{1}) were drenched in each pot at 25 mL pot^{-1}. A set of mixed application of rhizobia and *T. harzianum* was also kept for comparison. Six seeds of soybean were sown in each pot. Each treatment was replicated four times and pots were randomized on a screen house bench. Pots without amendment or biocontrol agent served as control. After germination, four seedlings were kept in each pot and excess were removed. After one week of seed germination, seedlings of each pot were inoculated with egg masses of *Meloidogyne javanica* of equal sizes at 10-egg masses pot^{-1}.

To assess the efficacy of mustard cake and biocontrol agents in suppression of root disease, plants were uprooted 6 weeks after nematode inoculation and roots were washed under tap water. Nematode infection was determined by counting the number of galls per root system. To determine nematode penetration and infection by root-infecting fungi, roots from each plant were cut into 1 cm long pieces and five pieces of tap roots from each plant were used for assessment of fungal infection. The remaining roots were mixed thoroughly, and a 1 gram sub-sample was wrapped in muslin cloth and dipped in boiling 0.25% acid fuchsin stain for 3-5 minutes. Roots were left in the stain to cool, and then washed under tap water to remove excess stain. Roots were transferred to vials containing glycerol and water (1:1 v:v) with a few drops of lactic acid. Roots were macerated in an electric blender for 45 seconds and the resulting suspension was suspended in 50 mL water. Numbers of juveniles and females in five 5 mL subsamples were counted with the aid of a low power microscope (6x) and number of nematodes g^{-1} root was calculated (Siddiqui and Ehteshamul-Haque, 2001). To determine the incidence of fungal infection, 1 cm long root pieces from tap roots (five pieces from each plant) were surface

disinfected with 1% $Ca(OCl)_2$ and plated onto potato dextrose agar amended with penicillin (100,000 units L^{-1}) and streptomycin (0.2 g L^{-1}). After incubation for 5 days at 28 °C, colonies of *M. phaseolina*, *R. solani* and *Fusarium* spp. were recorded. Data on plant height and fresh weight of shoots were also recorded.

Statistical analysis

The experiment was conducted twice and data were subjected to analysis of variance (ANOVA). For fungal infection, two-way ANOVA was used to compare the means among the treatments and also among different fungal pathogens. The follow-up of ANOVA included least significant difference (LSD) at ($p<0.05$) to compare the means, whereas for plant growth parameters and infection by the nematode, one-way ANOVA was used and LSD at ($p<0.05$) was calculated (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In vitro antifungal activity of rhizobia

Of the rhizobial strains/isolates in use, NFB-1(*Bradyrhizobium* sp.) inhibited all the four test fungi and produced a zone of inhibition of 10.6 mm against *F. solani*, 11.5 mm against *F. oxysporum*, 8 mm against *M. phaseolina* and 2 mm against *R. solani*, whereas NFB-2 (*Rhizobium* sp.), NFB-28 (*S. meliloti*) and NFB-29 (*S. meliloti*) inhibited at least three fungi (Table 1). Inhibition of root rotting fungi by rhizobia has been reported (Al-Ani et al., 2012; Noreen et al., 2016), and rhizobia can be use as a biocontrol agent against soil-borne plant pathogens as well as for biological nitrogen fixation (Brockwell et al., 1995).

In vitro nematicidal activity of rhizobia

Culture filtrates of the rhizobial strains also showed nematicidal effects, killing the second stage juveniles of *M. javanica* to varying degrees. Culture filtrates of NFB-1, NFB-2, NFB-28 and NFB-29 showed killing of 66%, 62%, 50% and 45%, respectively (Table 1). Among the plant parasitic nematodes, the root-knot nematodes are the most important phytonematodes from an economic perspective, as they parasitize more than 2000 plant species and cause 5% loss to agriculture worldwide (Barker et al., 1998; Shahina et al., 2009). There are reports of suppression of root knot nematode on chickpea by rhizobia (Noreen et al., 2016), which could be used for the control of root knot nematode as well as soil fungi (Qureshi et al., 2012) and fluorescent *Pseudomonas* (Noreen et al., 2015).

Screen house experiment

Infection of *M. phaseolina* was not found in treatments that used mustard cake at 1%, NFB-2, mustard cake at 1% +*T. harzianum*, mustard cake + NFB-1, mustard cake at 3% + NFB-28, mustard cake at 3% + NFB-29, *T. harzianum* + NFB-1, *T. harzianum* + NFB-2, *T. harzianum* + NFB-28 and

Table 1 - In vitro growth inhibition of Rhizoctonia solani, Fusarium solani F. oxysporum and *Macrophomina phaseolina* by rhizobial isolates in dual-culture plate assay

Rhizobial strain	*F. solani*	*R. solani*	*F. oxysporum*	*M. phaseolina*
	Zone of inhibition (mm)			
NFB-1 *Bradyrhizobium* sp.	10.6	2	11.5	8
NFB-2 *Rhizobium* sp.	12	2	5	*
NFB-28 *S. meliloti*	7.6	9.3	*	12.5
NFB-29 *S. meliloti*	10	3.6	*	5.5

* No inhibition. ** Growth inhibited but test fungus overgrows. *** Growth inhibited but no zone of inhibition formed.

T. harzianum + NFB-29. *Trichoderma harzianum* and rhizobial isolates NFB-1, NFB-28, NFB-29, also significantly suppressed *M. phaseolina* infection (Table 2). Application of mustard cake at 3%, *T. harzianum*, NFB-1, mustard cake + NFB-28, mustard cake + NFB-29, mustard cake + NFB-1, *T. harzianum* + NFB-1 resulted in complete suppression of *F. oxysporum*. Complete inhibition of *F. solani* infection was achieved by the application of *T. harzianum*, NFB-29, mustard cake + NFB-1, *T. harzianum* + NFB-1, *T. harzianum* + NFB-2, *T. harzianum* + NFB-28, and *T. harzianum* + NFB-29. Furthermore mustard cake at 3%, NFB-1, NFB-29, mustard cake at 1% + *T. harzianum*, mustard cake at 1% + NFB-28, mustard cake at 3% + NFB-28, mustard cake at 3% + NFB-29, also caused a significant reduction in *F. solani* infection (Table 3). In addition to a wide variety of organic matters that have been tested as organic amendments for managing plant pathogens, oil seed cakes suppressed soil-borne pathogens (Ehteshamul-Haque et al., 1995; Shafique et al., 2015; Rahman et al., 2016).

Table 2 - *In vitro* growth inhibition of root-infecting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* by cell free culture filtrates of rhizobial strains in agar disc-diffusion assay

Treatment	*M. phaseolina*	*R. solani*	*F. oxysporum*	*F. solani*
	Zone of Inhibition (mm)			
Control	0	0	0	0
NFB-1 (*Bradyrhizobium* sp.)	5	*	**	5
NFB-2 (*Rhizobium* spp.)	2.0	*	0	6.0
NFB-28 (*S. meliloti*)	0	0	0	8.3
NFB-29 (*S. meliloti*)	0	*	0	3

* No inhibition. ** Growth inhibited but no zone was formed.

Table 3 - Effect of cell free culture filtrates of different rhizobial isolates on juvenile mortality of *Meloidogyne javanica* by the root knot nematode after 48 hours

Rhizobial strains	Juvenile mortality %
Control (YMA broth)	01
NFB-1 (*Bradyrhizobium* sp.)	66
NFB-2 (*Rhizobium* sp.)	62
NFB-28 (*S. meliloti*)	50
NFB-29 (*S. meliloti*)	45

Amendment of soil with mustard cake or application of rhizobial isolates and *T. harzianum* showed a significant suppressive effect on root knot nematodes by reducing the number of galls per root system and endo-root nematode penetration. Application of rhizobial isolates NFB-1, NFB-2, NFB-28, and NFB-29 with mustard cake caused complete suppression of gall formation and nematode penetration in roots. Rhizobial isolate NFB-1 with *T. harzianum* and NFB-2 with mustard cake at 3% also caused complete suppression of nematode penetration resulting in gall free roots (Table 4). Greater plant height was caused by NFB-1 in use with mustard cake at 1%, whereas maximum fresh shoot weight was produced by NFB-2, and also when used with mustard cake at 3% (Table 5).

Oil seed cakes are by-products obtained after extraction of oil from the seeds. They are used as organic nitrogenous fertilizers because of to their NPK content. Some of these oil cakes are found to increase nitrogen uptake by plants and protect the latter from soil nematodes, insects, and parasites (Ramachandran et al., 2007). It was found that several antimicrobial by-products (e.g. organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds) are released during the decomposition of organic amendments, or synthesized by microorganisms involved in such degradation (Rodríguez-Kabanaet al., 1995). Mustard seed meal can be used for the

Table 4 - Combined effect of different species of rhizobia (NFB) and *Trichoderma harzianum* used as soil drench on infection of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum* and *F. solani* on soybean plants in soil amended with mustard cake (MC)

Treatment	*M. phaseolina*	*F. oxysporum*	*F. solani*
	Infection, %		
Control	25.0	75.0	37.5
Mustard cake (MC-1%)	00.0	6.25	18.7
Mustard cake (MC-3%)	25.0	00.0	6.20
T. harzianum	6.2	00.0	00.0
NFB-1 (*Bradyrhizobium* sp.)	6.2	00.0	12.5
NFB-2 (*Rhizobium* sp.)	00.0	6.20	31.5
NFB-28 (*S. meliloti*)	18.7	25.0	00.0
NFB-29 *S. meliloti*	18.7	6.2	6.2
MC-1% + *T. harzianum*	00.0	18.7	6.2
MC-1% + NFB-1 (*Bradyrhizobium* sp.)	00.0	00.0	00.0
MC-1% + NFB-2 (*Rhizobium* sp.)	6.2	00.0	31.2
MC-1% + NFB-28 (*S. meliloti*)	6.2	00.0	12.5
MC-1% + NFB-29 (*S. meliloti*)	00.0	00.0	43.7
MC-3% + *T. harzianum*	00.0	6.2	37.5
MC-3% + NFB-1 (*Bradyrhizobium* sp.)	6.2	00.0	37.5
MC-3% + NFB-2 (*Rhizobium* sp.)	6.2	12.5	25.0
MC-3% + NFB-28 (*S. meliloti*)	00.0	12.5	12.5
MC-3% + NFB-29 (*S. meliloti*)	00.0	12.5	12.5
T.harzianum+ NFB-1 (*Bradyrhizobium* sp.)	00.0	00.0	31.2
T.harzianum+ NFB-2 (*Rhizobia* sp.)	00.0	12.5	00.0
T.harzianum+ NFB-28 (*S. meliloti*)	00.0	37.5	00.0
T.harzianum+ NFB-29 (*S. meliloti*)	00.0	6.2	00.0

$LSD_{0.05}$ = Treatments = 14.5[(1)], Pathogens = 5.3[(2)].

[(1)] Mean values of treatments in columns showing difference of LSD values are significantly different at ($p<0.05$). [(2)] Mean values of pathogens in rows showing difference of LSD values are significantly different at ($p<0.05$).

Table 5 - Effect of different species of rhizobia (NFB) and *Trichoderma harzianum* in soil amended with mustard cake (MC) on the growth of soybean plants after 45 days

Treatment	Plant Height (cm)	Shoot fresh weight (g)	Galls/ root system	Juveniles & female/gm root
Control	16.0	0.90	3.87	20.7
MC-1%	19.80	1.07	0.81	9.0
MC-3%	27.18	1.59	0.87	9.25
T.harzianum	21.43	1.47	0.31	5.0
NFB-1 (*Bradyrhizobium* sp.)	27.12	1.14	1.75	7.25
NFB-2 (*Rhizobium* sp.)	20.30	0.84	0.13	0.75
NFB-28 (*S. meliloti*)	25.93	1.09	2.43	6.75
NFB-29 (*S. meliloti*)	26.75	1.45	0.50	00.0
MC-1% + *T. harzianum*	22.50	1.22	0.13	00.0
MC-1% + NFB-1 (*Bradyrhizobium*sp.)	30.06	1.40	00.0	00.0
MC-1% +NFB-2 (*Rhizobium* sp.)	13.75	0.64	00.0	00.0
MC-1% + NFB-28 (*S. meliloti*)	24.13	0.88	00.0	00.0
MC-1% + NFB-29 (*S. meliloti*)	17.75	0.67	00.0	00.0
MC-3% + *T. harzianum*	21.10	1.51	0.68	1.25
MC-3% +NFB-1 (*Bradyrhizobium* sp.)	27.80	1.11	0.81	1.75
MC-3% + NFB-2 (*Rhizobium* sp.)	28.17	1.95	00.0	00.0
MC-3% + NFB-28 (*S .meliloti*)	21.12	0.74	0.07	00.0
MC-3% + NFB-29 (*S. meliloti*)	21.60	1.13	0.25	1.0
T.harzianum + NFB-1(*Bradyrhizobium* sp.)	20.0	0.87	00.0	00.0
T.harzianum + NFB-2 (*Rhizobia* sp.)	70.0	1.15	0.43	1.25
T. harzianum + NFB-28(*S.meliloti*)	20.0	0.86	1.10	1.50
T. harzianum + NFB-29 (*S. meliloti*)	14.13	0.93	0.63	1.0
$LSD_{0.05}$	3.18[(1)]	0.78[(1)]	1.02[(1)]	1.47[(1)]

[(1)] Mean values of treatments in columns show a difference in LSD values that are significantly different at ($p<0.05$).

management of root knot nematodes (Meyer et al., 2015) while *T. harzianum* is a well-known biocontrol agent against root rot pathogens (Chet, 1987; Afzal et al., 2013). By contrast, rhizobia have gained attention for being used as biocontrol agents as well as nitrogen fixers (Deshwal et al., 2003; Noreen et al., 2016).

Rhizobia and *Trichoderma harzianum* can be used as biocontrol agents against root rotting fungi *viz; M. phaseolina*, *R. solani*, *F. solani* and *F. oxysporum* as well as root knot nematode *M. javanica.* Mustard oil cake alone or mixed with a biocontrol agent could be developed for management of soil-borne diseases of soybean.

ACKNOWLEDGMENTS

Sincere thanks are expressed to Prof. Dr. Riaz Ahmad, University of Oklahoma, Stillwater, OK, USA and Dr. Ozair Chaudhry, Albert Campbell Collegiate Institute (NS) Scarborough, Ontario, Canada and Prof. Dr. Seema Mahmood, University of Glasgow, Glasgow, Scotland UK for their critical comments and valuable suggestions on the manuscript. Financial assistance of the Higher Education Commission, Islamabd is greatfuly acknowledged.

REFERENCES

Afzal S. et al. Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. **Pak J Bot.** 2013;45:1455-60

Al Ani R. et al. *Rhizobium japonicum* as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina.* **Plant Prot Sci.** 2012;48:149-55.

Aziz A., Al-Aska A. In vitro antifungal activity of three Saudi plants extracts against some phytopathogenic fungi. **J Plant Prot Res.** 2012;52(4):458-62.

Bailey K.L., Lazarovits G. Suppressing soil-borne diseases with residue management and organic amendments. **Soil Till Res.** 2003;72:169-80.Tab

Barker K. Introduction and synopsis of advancements in nematology. In: Barker K.R. et al., editors. **Plant nematode interactions**. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 1998. p.1–20.

Brockwell J., Bottomly P.J., Thies J.E. Manipulation of rhizobia microflora for improving legumes productivity and soil fertility: a critical assessment. **Plant Soil**. 1995;174:143-80.

Chet I. Trichoderma-application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Chet I., editor. **Innovative approaches to plant disease control**. New York: John Wiley and Sons, 1987. p.147-60.

Deshwal V.K. et al. Rhizobia as a biological control agent against soil borne plant pathogenic fungi. **Ind J Exp Biol.** 2003;41:1160-4.

Ehteshamul-Haque S., Ghaffar A. Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. **J Phytopathol.** 1993;138:157-63.

Ehteshamul-Haque S., Abid M., Ghaffar A. Efficacy *of Bradyrhizobium* sp. and *Paecilomyces lilacinus* with oil cakes in the control of root rot of mungbean. **Trop Sci.** 1995;35:294-9.

Friedman M., Brandon D.L. Nutritional and health benefits of soy proteins. **J Agric Food Chem.** 2001;49:1069-86.

Garbeva P., van Veen J.A., van Elsas J.D. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. **Ann Rev Phytopathol.** 2004;42:243-70.

Gomez K.A., Gomez A.A. Statistical procedures for agricultural research. 2nd ed. New York: Wiley, 1984.

Ji S.H., Gururani M.A., Chun S.C. Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from korean rice cultivars. **Microbiol Res.** 2014;169:83-98.

Khalil M.E.H., Allam A., Barakat A.T. Nematicidal activity of some biopesticides and microorganisms against root- knot nematode on tomato plants under greenhouse conditions. **J Plant Protec Res.** 2012;52(1):47-52.

Keen, B.A., Raczkowski H. Relation between the clay content and certain physical properties of a soil. **Journal of Agricultural Science**. 1921; 11: 441-449.

Meyer S.L.F. et al. Mustard seed meal fo management of root-knot nematode and weeds in tomato production. **HortTechnology**. 2015;25:1992-202**.**

Nautiyal C.S. Rhizosphere competence of *Pseudomonas* sp., NBR 19926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. **FEMS Microbiol Ecol.** 1997;23:145-58.

Neeraj S.S. et al. Efficient nitrogen fixing rhizobial isolate infecting *Vigna radiata* L. **Asian J Agric Sci.** 2009;1(2):62-5.

Noreen R. et al. Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. **Crop Protect.** 2015;75:18-24

Noreen R. et al. Role of mungbean root nodule associated fluorescent *Pseudomonas* and rhizobia in suppressing the root rotting fungi and root knot nematode affecting chickpea (*Cicer arietinum* L.). **Pak J Bot.** 2016;48:2139-45.

Ozkoc I., Deliveli M.H. In vitro inhibition of mycelial growth of some root rot fungi by *Rhizobium leguminosarum* biovar *phaseoli.* **Turk J Biol.** 2001;25:435-45.

Qureshi S.A. et al. Nematicidal potential of culture filtrates of soil fungi associated with rhizosphere and rhizoplane of cultivated and wild plants. **Pak J Bot.** 2012;44:1041-6.

Rafi H., Dawar S., Tariq M. Combined effect of soil amendment with oil cakes and seed priming in the control of root rot fungi of leguminous and non-leguminous crops. **Pak J Bot.** 2016;48:1305-11.

Rahman A. et al. Induction of systemic resistance in cotton by the neem cake and *Pseudomonas aeruginosa* under salinity stress and *Macrophomina phaseolina* infection. **Pak J Bot.** 2016;48:1681-9

Ramachandran K. et al. Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (*Piper nigrum* L) cuttings. **Develop Plant Soil Sci.** 2007;102:324-31.

Rodríguez-Kábana R et al. Mixtures of olive pomace with different nitrogen sources for the control of *Meloidogyne* spp. on tomato. Supplement to the **J Nematol.** 1995;27(4S):575-84.

Siddiqui I.A., Ehtheshamul-Haque S. Suppression of root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: the influence of inoculums density, nematode population, moisture, and other plant associated bacteria. **Plant Soil.** 2001;237:81-9.

Shafique H.A. et al. Role of antagonistic microorganisms and organic amendment in stimulating the defense system of okra against root rotting fungi. **Pol J Microbiol.** 2015;64:155-60

Shahina F. et al. Research on rootknot nematode Meloidogyne by Pakistani scientists: A review with description of species prevalent in Pakistan. **Pak J Nematol**. 2009;27:1-115.

Simpfendorfer S., Harden T.J., Murray G.M. The in vitro inhibition of *Phytophthora clandestine* by some rhizobia and the possible role of *Rhizoium trifolii* in biological control of *Phytophthora* root rot of subterranean clover. **Aust J Agric Res.** 1999;50:1469-73.

Spaink H.P. Root nodulation and infection factors produced by rhizoial bacteria. **Ann Rev Microbiol.** 2000;54:257-88.

Spaink H.P. et al. Novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host speciûcity of *Rhizobium.* **Nature.** 1991;354:125-30.

Sprent J.I. Nodulation in legumes. Kew: Royal Botanical Gardens, 2001.

Vasileva V., Ilieva A. Nodulation and nitrogen assimilation in legumes under elements of technology. Lap Lambert Academic Publishing, 2012. 120p.

Yang X.B., Feng F. Ranges and diversity of soybean fungal diseases in North America. **Phytopathology**. 2001;91:769-75.