Pak. J. Bot., 44(6): 2077-2080, 2012.

INCIDENCE OF ROOT ROT DISEASES OF SOYBEAN IN MULTAN PAKISTAN AND ITS MANAGEMENT BY THE USE OF PLANT GROWTH PROMOTING RHIZOBACTERIA

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Abstract

Eight villages in Multan district were surveyed to record incidence of disease and losses of soybean (*Glycine max* L.) caused by root rot fungi. The root incidence ranged 10-17% and losses ranged 6.75-15.5%. The evaluation of four PGPR isolates was used in combination with organic amendment for the management of root-rot disease incidence and to reduce the population of root pathogenic fungi and to increase the yield in field. This study demonstrated effective biological control by the PGPR isolates tested, thereby indicating the possibility of application of rhizobacteria for control of soil borne diseases of soybean in Pakistan and other countries.

Introduction

Soybean (*Glycine max* L.) is important nitrogen fixing leguminous crop cultivated for food and feed. Soybean oil, soymilk and soy meal are important products of soybean used by human beings. This crop is prone to attack by different pathogens, including fungi, bacteria, nematode and virus. Among all these pathogens the most destructive pathogen for this crop is fungus. It causes heavy yield losses of this crop every year. The root rot pathogenic fungi are major threat for this crop as these fungi attack on the root of the plant and destroy the proper functioning of the plant to take water and other nutrients upward. *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. are considered as major soybean seedling pathogens, which contribute to stand reduction. In Pakistan, many soil-borne root-infecting fungi attack soybean crops, and vastly reduce yield (Ehteshamul-Haque & Ghaffar, 1994). Our important potential oilseed crops like, sunflower and soybean are prone to attack by root infecting fungi like *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Pythium* spp., and *Rhizoctonia solani* (Ehteshamul-Haque, 1994; Ehteshamul-Haque & Ghaffar. 1993; Inam-ul-Haq *et al*., 2003; Dieter & Défago, 2005).

As agricultural production intensified over the past few decades, growers became more and more dependent on agrochemicals as a reliable method of crop protection. However increased use of chemicals caused several negative effects for example development of pathogen resistance to applied chemicals and their non-target environmental impacts. Moreover increasing cost of pesticides, particularly in developing countries and search for pesticide free food in developed countries has led to a search for substitutes for these products. Moreover there are many diseases in which chemical solutions are ineffective. Biological control is thus being considered as an alternative way of the chemical management in agriculture. It is defined as the practice of using beneficial natural organisms to attack and control harmful plant and animal pests and weeds, called biological control.

Plant growth promoting rhizobacteria (PGPR) were first defined by Kloepper & Schroth, (1978) as the soil bacteria that are closely associated with plant roots, colonize them and enhance plant growth. Associated microorganisms in soil environment influence plant diseases. Interaction of one pathogen may also alter the host response to subsequent infection by another (Taylor, 1979). Infection of roots by soil-borne root infecting fungi resulting in development of root rot and wilt diseases (Armstrong *et al*., 1976) and one fungus predispose crop plant to infection by other fungi (Dieter & Défago, 2005). The prospects of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and greenhouse studies (Dawar *et al.*, 2008), but responses have been variable in the field (Bowen & Rovira,1999).

The use of organic matter in the soil is an old practice considered essential for sustaining the thriftiness and productivity of crop. In addition to providing necessary nutrient elements, organic matter also influences soil physical characters such as pore size, aeration, temperature, water retention capacities etc. These help in better solubilization of minerals which together with the nutrients released by decomposing matter help in rapid extension of the root system, better uptake of nutrients, retention of added nitrogen for a longer period and finally better plant vigor even if the disease has not been significantly checked (Singh, 1983). Neem plant (*Azadirachta indica*) possesses biologically active compounds, mainly alkaloids such as isoprenoids that control various pests including fungi (Anwar-ul-Haq, 1993).

Keeping in view the beneficial effects of rhizobacteria and use of organic amendments, to control the soil borne diseases of soybean crop, the objective of the present study was to evaluate four rhizobacterial isolates and to find the best one among these. These four rhizobacterial isolates were taken up from the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, and neem cake for the management of root rot diseases of soybean caused by soil borne fungi.

Materials and Methods

A comprehensives survey was carried out in eight different localities in Multan district and disease incidence and loss assessment due to root infecting fungi was calculated. Sampling of the diseased plants was done by uprooting the whole plant from soil with the help of a spade. Effort was made to take out the entire root system by digging the roots carefully. After excising the aerial portion and removing soil from the root system of the uprooted plants, the roots were placed in polyethylene bags. All the bags were tied and labeled. Three to four samples were taken from a single site. Rhizosphere soil/nodule samples were collected from the same site.

Determination of disease incidence: The diseased plant that was under the attack of root infecting fungi, showing symptoms at the collar portion or wilt like symptoms was noted for the determination of disease incidence. In a field, total 50 plants were selected randomly. Out of these 50 plants, number of diseased plants was determined. After that the disease incidence was calculated by the following formula:

$$\text{Disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \times 100$$

Determination of yield losses % age: The percentage of yield losses caused by root infecting fungi was determined by the following formula:

$$\text{Losses \% age} = \frac{\text{Obtained yield (diseased plants)}}{\text{Standard yield of healthy plants}} \times 100$$

Isolation of fungi

a. Isolation of *Fusarium solani*: Soil dilution technique was used for the isolation of *Fusarium solani*. One gram of soil sample was suspended in 9 ml of 0.1% agar suspension instead of plain water and dilution series was made. One ml of aliquot of 0.1% agar suspension from the final soil dilution was poured on Petri dishes containing PCNB and the suspension spread on agar surface by rotating the dishes. Plates were inoculated at 28°C for five days and species of *Fusarium* were identified after reference to Nash & Snyder, (1962).

b. Isolation of *Rhizoctonia solani*: Baiting technique was used for the isolation of *Rhizoctonia solani*. Sterilized sorghum strains were used as baits and placed on the moist soil surface. The baits were removed after 24 hours, washed in tap water and transferred on PDA, pH 5.5, for growth and identification of *Rhizoctonia solani* (Wilhelm, 1955).

c. Isolation of *Macrophomina phaseolina*: Wet sieving and dilution technique as suggested by Sheikh & Ghaffar (1975) was used. Twenty gram soil sample was wet sieved through 100 mesh (150 um) placed on 300-mesh (53 um) screen. The residue obtained on 53 um screen was washed in running tap water for one minute and transferred into a beaker containing 0.5% Ca $(OCL)_2$ and made up to 100 ml to produce 1:5 dilution. The sclerotial suspension was put on a magnetic stirrer and 1 ml aliquot was evenly spread on to the surface of PDA plates containing penicillin (100,000 units/l) and streptomycin (0.2 g/l), Demosan 0.3 gm/litre and rose Bengal 0.1 g/l. The plates were incubated at 28°C and in five days grayish to black colonies of *M. phaseolina* were identified.

$$\text{Infection \%} = \frac{\text{No. of healthy plants} - \text{No. of plants infected by a pathogen}}{\text{Total No. of healthy plants}} \times 100$$

$$\text{Colonization \%} = \frac{\text{No. of root pieces colonized by a pathogen}}{\text{Total No. of root pieces of all plants}} \times 100$$

Population of PGPR

a. Colony forming units (cfu) per ml in suspension: After making the suspension of biological antagonists, population of bacteria, the dilution plate method was used. One ml suspension was poured on nutrient agar medium and incubated at 28°C for 3-7 days. Bacteria growing on plates were counted and multiplied by the dilution factor, which gave cfu/ml of bacteria.

b. Colony forming unit (cfu) per seed: Ten seeds after treatment with suspension of microbial antagonists were transferred in test tubes containing 10 ml sterilized water. The test tube was shaken and dilution series was made. A dilution plate method was used for the bacterial colony forming units of bacteria which was calculated by using the following formula:

$$\text{cfu of bacteria per seed} = \text{No. of colonies of bacteria} \times \text{dilution factor}$$

Green house experiment: Soybean hybrid seeds taken up from the Oil Seed Section in Ayub Agriculture Research Institute, Faisalabad, were treated with 1 % gum arabic suspension of *P. fluorescens* strains S-22 (6×10^7 cfu ml^{-1}), S-19 (3.5×10^7 cfu ml^{-1}) S-26 (3.7×10^7 cfu ml^{-1}) and S-17 (1×10^7 cfu ml^{-1}). Eight seeds were sown in 15 cm diameter earthen pots, each containing 1.25 kg naturally infested soil taken from the research area of the Department of Plant Pathology, University of Agriculture, Faisalabad. The treatments were as follows:

T1 Control
T2 Neem Cake (NC)
T3 S17 + NC
T4 S19 + NC
T5 S22 + NC
T6 S26 + NC
T7 S17 + S19 + NC
T8 S17 + S22 + NC
T9 S17 + S26 + NC
T10 S19 + S22 + NC
T11 S19 + S26 + NC

Each treatment was replicated three times. Untreated seeds served as control. The soil already had a natural infestation of 3-9 sclerotia of *M. phaseolina* per gram of soil, 4-11% colonization of *R. solani* on sorghum seeds used as baits and 3500 cfu per gram of *F. solani*. The neem cake was applied @ 0.2% weight of the soil. The pots were arranged in a completely randomized block design in the green house. The temperature during the growth period ranged from 22-29^{o}C. After germination seedlings were thinned to four seedlings per pot, and pots were kept at 52% water holding capacity.

Incidence of root infecting fungi was determined by the modified method of Short *et al*., (1980). Plants were uprooted after 6 weeks growth. Roots were washed and pieces of 1 cm were made from each plant. The root pieces were surface sterilized with 1% Ca (OCl) $_2$ for 3 min and were transferred onto potato dextrose plates containing penicillin (100,000 Ul^{-1}) and streptomycin (200 mg l^{-1}). The incidence of root-infecting fungi (*Fusarium* spp. *M. phaseolina* and *R. solani*) was recorded and data for each pathogen was analyzed by F-test and means were separated by DMR Test at alpha 0.05.

Results and Discussion

Use of different strains of *P. aeruginosa* significantly ($p<0.05$) reduced infection of root-infecting fungi and enhanced plant growth. Mansoor *et al*., (2007) and several other researchers have also observed the suppression of root fungi by *P. aeruginosa*. Root colonizing bacteria that have a beneficial effect on plants have been reported to improve plant growth either through direct stimulation of the plant, by producing growth regulators (Naveed *et al.,* 2008), or suppressing the pathogens (Raaijmakers *et al*., 2002). Of the various rhizospheric bacteria, those belonging to the fluorescent *Pseudomonas* spp., which colonize roots of a wide range of crops are reported to be antagonistic to soil borne pathogens (Siddiqui *et al*., 2000). The production of certain antibiotics and siderophores by *P. aeruginosa* has been regarded as one of the mechanisms involved in this antagonism (Levy *et al*., 1992). Raaijmakers & Weller (1998) described the role of 2, 4-diacetylphloreglucinol, an antifungal metabolite from species of fluorescent *Pseudomonas* in root disease suppression. Van Peer *et al*., (1991) observed induced resistance in carnation against *Fusarium* wilt by a strain of *Pseudomonas* sp.

From all the ten treatments the calculated infection % due to root rot fungi, *M. phaseolina, R. solani* and *F. solani*, showed decrease in case of T3 and T4. When these rhizobacterial isolates were used along with neem cake they were highly effective against *M. phaseolina* and *Rhizoctonia solani* (Table 2). When this data was subjected to statistical analysis it was clear that the isolates S17 and S19 were highly effective individually as well as with neem cake (NC). From this study we conclude that soybean fields located at Momanabad had highest level of root rot disease (Table 1). PGPR S-17 and S-19 effectively reduced the disease incidence. Addition of neem cake enhanced the effectiveness of PGPR.

Table 1. Incidence and yield loss of soybean crop at eight localities in Multan district.

S. No.	Name of village	Variety	Disease incidence (%)	Loss (%)
1.	Baghwala	NARC-2	10	10
2.	Laar	NARC-2	11	11.50
3.	Ch# 7 Faiz	NARC-2	17	15.50
4.	Baseera	FS-85	11	11
5.	Ch# 5 Faiz	Williams-82	06	06.75
6.	Qazi wala	Williams-82	12	11.50
7.	Basti Malook	FS-85	10	09.25
8.	Mominabad	NARC-1	12	11.50

Table 2. Effect of PGPR strains as seed dressing on the infection % of root rot fungi.

Treatments	Disease insidence				
	M. phaseolina	***R. solani***	***F. oxysporum***	***F. solani***	***Pythium* spp.**
Control	72 a	63 a	58 a	48 a	58 a
Neem Cake	65 b	57 b	50 b	42 bc	52 b
S-22	63 b	61 a	49 b	43 b	51 b
S-19	52 c	46 d	42 d	32 d	36 d
S-17	44 e	42 e	38 e	32 d	32 e
S-26	65 b	64 a	49 b	42 bc	54 b
S-22 + Neem Cake	48 d	51 c	45 c	41 bc	46 c
S-19 + Neem Cake	10 f	3 f	5 f	6 e	0 f
S-17 + Neem Cake	5 g	3 e	4 f	6 e	3 f
S-26 + Neem Cake	44 e	42 e	39 e	40 c	38 d

*Means with the same letters are not significantly different from each other at p = 0.05
According to Duncan's Multiple Range Test (DMR)

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(Received for publication 27 July 2011)