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BURKHOLDERIA SP. STRAIN TNAU-1 FOR BIOLOGICAL CONTROL OF ROOT ROT IN MUNG BEAN (*VIGNA RADIATA* L.) CAUSED BY *MACROPHOMINA PHASEOLINA*

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Abstract: The potential of *Burkholderia* sp. strain TNAU-1 for the management of mung bean (*Vigna radiata* L.) root rot caused by *Macrophomina phaseolina* was evaluated under greenhouse conditions. *Burkholderia* sp. strain TNAU-1 inhibited the mycelial growth of *M. phaseolina in vitro* and produced an inhibition zone of 18.8 mm. Mung bean seeds when treated with the bacterial suspension, showed significant increase in root length, shoot length and seedling vigour. A talc-based powder formulation of *Burkholderia* sp. strain TNAU-1 was developed and evaluated for its efficacy in the management of mung bean root rot under greenhouse conditions. Seed treatment or soil application of the powder formulation of *Burkholderia* sp. strain TNAU-1 significantly reduced the incidence of root rot and increased the germination percentage and plant height. Seed treatment with the powder formulation of *Burkholderia* sp. strain TNAU-1 alone was effective in controlling root rot disease; but the combined seed treatment and soil application of *Burkholderia* sp. strain TNAU-1, increased the efficacy. Seed treatment and soil application with *Burkholderia* sp. reduced the root rot incidence from 52.6 per cent (with non-bacterized seeds) to 16.7 per cent. Control of root rot with the application of *Burkholderia* sp. by seed treatment and soil application was not statistically different from that obtained with seed treatment with carbendazim. The endophytic movement of *Burkholderia* sp. in the stem, roots and leaves of mung bean was confirmed through PCR using *Burkholderia* sp. specific primers which resulted in the amplification of a 417 bp product.

Key words: biocontrol, *Burkholderia* sp., *Macrophomina phaseolina*, mung bean

INTRODUCTION

Mung bean or Green gram *Vigna radiata* (L.) Wilczek (syn: *Phaseolus aureus* Roxb.) constitutes the important group of grain legumes which form a major source of dietary proteins of high biological value, energy, minerals and vitamins (Taylor *et al*. 2005). However, the yield of mung bean is greatly reduced due to various factors of which diseases caused by fungi and viruses are of major concern. Of these the root rot disease caused by the soil borne fungus *Macrophomina phaseolina* (Tassi) Goid is a major limiting factor in the mung bean production causing considerable losses (Raguchander *et al*. 1993). The fungus *M. phaseolina* infects more than 500 plant species worldwide (Wyllie 1993) and causes charcoal rot disease in several agronomically important crops including soybean, maize, sorghum and cotton. Variation in virulence and morphological characters among isolates of *M. phaseolina* have been reported (Dhingra and Sinclair 1978). Su *et al*. (2001), while investigating the genetic variability among *M. phaseolina* isolates from soybean, corn, sorghum and cotton by RFLP and RAPD analyses, observed no variations among isolates and hence the authors concluded that *M. phaseolina* constitutes a single species. Presently, there are no commercial mung bean cultivars that are resistant to *M. phaseolina*. Disease management strategies primarily depend on seed treatment and soil drenching with fungicides. Though some of the fungicides are found effective in inhibiting the growth of *M. phaseolina in vitro*, soil drenching with fungicides is neither practical nor economical. Seed treatment with fungicides also does not protect the crop for a long period. Furthermore, the usage of fungicides produces a negative impact on nodulation of legumes by nitrogen fixing beneficial bacteria (Muthomi *et al*. 2007). Increasing awareness on the deleterious effects of indiscriminate usage of fungicides on the environment, on human beings and animals has led to a search for alternatives to control root rot disease. The use of antagonistic plant growth-promoting bacteria has been realized in the recent past. Such bacteria offers great potential as alternatives to chemical pesticides. The specific inherent qualities of the antagonistic bacteria are competition, antibiosis and plant growth promotion. A number of biocontrol agents such as *Trichoderma* sp. (Raguchander *et al*. 1993; Rettinas-

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sababady *et al*. 2002; Indira and Gayatri 2003), and *Pseudomonas fluorescens* (Karthikeyan *et al*. 2005; Saravanakumar *et al*. 2007; Thilagavathi *et al*. 2007) have been used to control root rot of pulses. Recently, we reported that seed treatment at 10 g/kg, or soil application at 2.5 kg/ha 30, 45, 60 days after sowing with the formulation of *Burkholderia* sp. strain TNAU-1 significantly reduced *Aspergillus flavus* infection and aflatoxin B1 contamination in peanut kernels (Vijayasamundeeswari *et al*. 2010). This bacterial strain also shows *in vitro* antagonistic activity against several agronomically important plant pathogens.

In this investigation the potential of *Burkholderia* sp. strain TNAU-1 in the management of dry root rot disease of mung bean was evaluated under greenhouse conditions.

MATERIALS AND METHODS

Fungal and bacterial cultures

The fungus, *M. phaseolina* was isolated from a root rot-infected mung bean (*V. mungo*) plant and maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) medium under laboratory conditions. *Burkholderia* sp. strain TNAU-1 was obtained from the culture collection of the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and used in this study.

In vitro* screening of *Burkholderia* sp. strain TNAU-1 against *M. phaseolina

The *Burkholderia* sp. strain TNAU-1 was tested for its ability to inhibit mycelial growth of *M. phaseolina* following the dual culture technique (Dennis and Webster 1971). *Burkholderia* sp. was streaked on one side of a Petri dish containing PDA medium at 1 cm from the edge of plate. The mycelial disc (8-mm-dia) from a 7-day-old culture of the fungal pathogen was placed on the opposite side in the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature (28± 2°C) for seven days. The growth of the fungus was inhibited when it grew towards the bacterial colony on PDA. The inhibition zone was measured from the edge of the test fungal mycelium to the edge of the bacterial colony.

Efficacy of *Burkholderia* sp. on plant growth

Seed bacterization

Burkholderia sp. strain TNAU-1 was grown on Potato Dextrose (PD) broth with constant shaking at 150 rpm for 48 h at room temperature (28±2°C). The bacterial cells were harvested by centrifugation at 6,000 rpm for 15 min and resuspended in 0.01M phosphate buffer (pH 7.0). The final concentration was adjusted to approximately 10^8 cfu/ml (OD_{595} = 0.3) in a spectrophotometer and used as inoculum (Thompson 1996). Required quantities of mung bean seeds (cv. Co-6) were soaked in the bacterial inoculum and dried in shade for 2 h.

Plant growth promotion

The plant growth promoting activity of *Burkholderia* sp. strain TNAU-1 was assessed based on the seedling vigour following the standard roll towel method (International Seed Testing Association 1996). The treated seeds were placed on coarse blotter paper sheets and covered with a moistened blotter and rolled. The roll was kept on a butter paper sheet and rolled as a bundle, and incubated in a growth chamber at 25°C with 80% RH. Four replications were maintained for each treatment. The root and shoot length of seedlings were measured and the germination percentage was calculated after 15 days. The vigour index was calculated as suggested by Baki and Anderson (1973).

Vigour index = per cent germination x seedling length (shoot length + root length)

Greenhouse experiments

The talc based formulation of *Burkholderia* sp. strain TNAU-1 was assessed for its efficacy in controlling root rot of greengram, under greenhouse conditions. The susceptible mung bean cultivar, cv. Co-6 was obtained from the Pulses Research Station, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Talc-based powder formulation of *Burkholderia* sp. strain TNAU-1 was developed as described by Vijaysamundeeswari *et al*. (2010). *M. phaseolina* was multiplied in sand- maize medium (Riker and Riker 1936) for 15 days and the sand-maize inoculum was mixed with a potting mixture at the rate of 10%. Mung bean seeds were sown after a week. Five seeds were sown in each pot, and five pots were kept as one replication. The trial was conducted in a completely randomized design (CRD) with four replications. The treatments were as follows T1: Seed treatment with talc-based formulation of *Burkholderia* sp. strain TNAU-1 at a rate of 10 g/kg; T2: Soil application with talc-based formulation of *Burkholderia* sp. strain TNAU-1 at a rate of 5 g/pot; T3: Combination of seed treatment (10 g/kg) and soil application (5 g/pot) with talc-based formulation of *Burkholderia* sp.; T4: Seed treatment with carbendazim at a rate of 2 g/kg and T5: The untreated control. Seedling emergence and disease incidence were recorded 20 days after sowing (DAS) and 30 DAS. Plant height was recorded at 30 DAS. The experiment was repeated three times. Arc sine transformation of data on percentage of root rot incidence was done. Duncan's multiple range test (DMRT) was first applied to the transformed values and then transferred to the original means (Gomez and Gomez 1984). The package used for analysis was IRRISTAT version 92-1 developed by the Internationl Rice Research Institute, Biometrics Unit, The Philippines.

Testing endophytic movement of Burkholderia sp. strain TNAU-1 in mung bean

The endophytic movement of *Burkholderia* sp. strain TNAU-1 in mung bean, applied through seed treatment or soil application, was studied following the method of Rajendran and Samiyappan (2008). Whole plants from both the treatment and the control were manually uprooted 21 days after treatment and brought to the laboratory. Root, stem and leaf sections (2–3 cm long) were made using a sterile scalpel. Plant samples were surface sterilized with 1% sodium hypochlorite (NaOCl) in 0.05% Triton X-100 for 10 min and rinsed four times in sterile

0.02 M potassium phosphate buffer (PB) pH 7.0. A 0.1 ml aliquot was taken from the final buffer wash and transferred to 9.9 ml potato dextrose broth to serve as a sterility check. Samples were discarded if growth was detected in the sterility check samples within 48 h. Each sample (0.5 g) was triturated with a sterile mortar and pestle in 9.5 ml of the final buffer wash. Serial dilutions up to 10^8 of the triturate were made in PB. Each dilution of every sample (0.1 ml) was plated on a Petri dish containing PDA medium. The plates were incubated at 28±2°C for 48 h. After incubation, the individual bacterial colonies developed in the plates were analyzed by colony PCR for confirmation of *Burkholderia* sp., using specific primers as described below.

Detection of Burkholderia sp. by colony PCR

Burkholderia sp. specific forward primer (5′ CGAACGGGTGAGTAATAC 3′) and reverse primer (5′ GCTGGCACGTAGTTAGC3′) for PCR assays were designed based on the nucleotide sequence (GenBank accession number EU560426) and synthesized by Operon Company (Operon Biotechnologies, Cologne, Germany) in salt free status. PCR was undertaken in 20 µl volume consisting of 5 mM each dNTPs, 20 pmol of each primers, 0.5 U of Taq DNA polymerase and single loop of bacterial colony. The reaction was carried out in a Eppendorf Master Cycler ep-gradient S (Eppendorf, A G, Hamburg, Germany) programmed with initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min. Following amplification, 10 µl of each PCR product was electrophoresed on 1.5% agarose gel in TAE buffer. To visualize DNA, gels were stained with ethidium bromide (0.1 mg/l) and then photographed under transmitted ultraviolet light using an AlphaImager 2000 (Alpha Innotech, San Leandro, CA, USA).

RESULTS AND DISCUSSION

The results indicated that *Burkholderia* sp. strain TNAU-1 inhibited the mycelia growth of *M. phaseolina in vitro* and produced an inhibition zone of 18.8 mm (Fig. 1). Mung bean seeds when treated with *Burkholderia* sp. showed significant increases in per cent germination, root length, shoot length and seedling vigour in the standard roll towel method (Table 1).

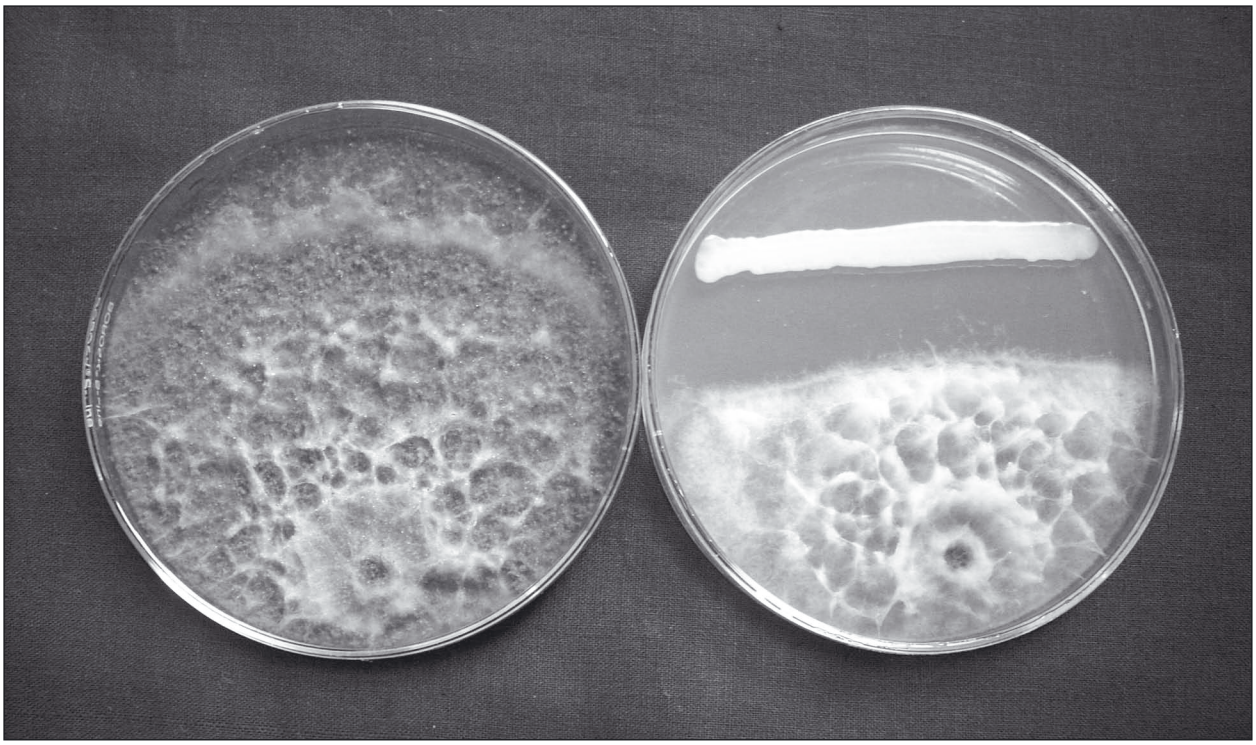


Fig. 1. Antagonistic activity of *Burkholderia* sp. strain TNAU-1 against *M. phaseolina*

A talc-based powder formulation of *Burkholderia* sp. strain TNAU-1 was prepared and tested for its plant growth promoting activity and efficacy in controlling root rot of mung bean under greenhouse conditions. The results of the greenhouse experiments indicated that seed treatment or soil application of powder formulations of *Burkholderia* sp. strain TNAU-1 significantly reduced the incidence of root rot and increased the per cent germination and plant height (Table 2). Seed treatment with the powder formulation of *Burkholderia* sp. strain TNAU-1 alone was effective in controlling root rot disease; but combined application with seed and soil of *Burkholderia* sp. strain TNAU-1 formulation increased the efficacy. Maximum reduction in disease incidence and enhancement of the plant height was noticed due to seed treatment and soil application with *Burkholderia* sp. under greenhouse conditions. Control of root rot with application of *Burkholderia* sp. strain TNAU-1 formulation by seed treatment and soil application was not statistically different from that obtained with seed treatment with

Table 1. Effect of seed bacterization with the formulation of *Burkholderia* sp. strain TNAU-1 on seed germination and seedling vigour of mung bean (cv. CO-6)

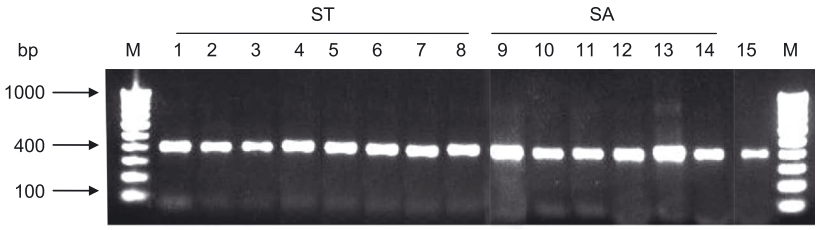
Treatments	Germination [%]	Shoot length [cm]	Root length [cm]	Vigour index
Burkholderia sp.	99 a	14.76 a	4.26 a	1901.75 a
Control	96 b	13.49 b	3.44 b	1692.25 b
CD (0.05)	1.98	0.65	1.86	240.51

Data are mean of four replications; data followed by the same letter in a column are not significantly different (p = 0.05) from each other according to Ducan's multiple rang test (DMRT)

Table 2. Effect of seed treatment or soil application with the formulation of *Burkholderia* sp. strain TNAU-1 against root rot of mung bean (cv. CO-6) caused by *M. phaseolina* under greenhouse conditions

Treatment	Germination [%]*	Plant height [cm]	Root rot incidence [%]**
Seed treatment	99.0 a	34.0 b	19.6 ab
Soil application	98.0 a	33.8 b	22.4 b
Seed treatment + Soil application	99.5 a	35.9 a	16.7 a
Seed treatment with carbendazim (2 g/kg)	99.5 a	27.3 c	17.1 a
Untreated Control	96.0 b	22.7 d	52.6 c
CD (0.05)	1.9	1.5	3.1

**Burkholderia* sp. strain TNAU-1 was applied as seed treatment (10 mg/g seed) or soil application (5 g/pot) or as seed treatment followed by soil application at the time of sowing; **arc sine transformation of data was done prior to analysis; the data are mean of three replications; means within a column followed by a common letter are not significantly different (p = 0.05) by DMRT



Lane M, 100 bp Marker; Lanes 1,2,3,9,10, endophytic bacteria from roots; Lanes 4,5,6,11,12, endophytic bacteria from stem; Lanes 7,8,13,14, endophytic bacteria from leaves, Lane 15, DNA from *Burkholderia* sp. strain TNAU-1(+ ve control)

Fig. 2. Endophytic movement of *Burkholderia* sp. strain TNAU-1 in mung bean

carbendazim. The endophytic movement of *Burkholderia* sp. strain TNAU-1 in the stem, roots and leaves of mung bean was confirmed through colony PCR using *Burkholderia* sp. specific primers, which resulted in the amplification of a 417 bp product (Fig. 2).

Biological control of soilborne pathogens offers a promising alternative to synthetic pesticides, in part because it is perceived as safe for the environment and the consumers of the plants that it protects (Heungens and Parke 2000). A number of biocontrol agents such as *Trichoderma viride* (Raghuchander *et al*. 1993; Indira and Gayatri 2003), *Pseudomonas fluorescens* (Thilagavathi *et al*. 2007) have been used to control root rot of pulses. Indira and Gayatri (2003) reported that the incidence of root rot in blackgram was significantly reduced by 50% when treated with *Trichoderma* spp. alone or in combination with the biofertilizer *Rhizobium*, both under glasshouse and field conditions. Raghuchander *et al*. (1993) demonstrated that *Trichoderma* isolates multiplied in organic substances, such as coir pith, groundnut shell and pressmud reducing the root rot caused by *M. phaseolina* in mungbean. Thilagavathi *et al*. (2007) reported that a combination of *P. fluorescens* strain Pf1+ *Trichoderma viride* strain Tv1 was most effective in reducing root rot of greengram caused by *M. phaseolina* under glasshouse and field conditions. Species belonging to the genus *Burkholderia* have been widely used as biocontrol agents against many phytopathogenic fungi, such as *Pythium aphanidermatum*, *Pythium ultimum*, *Fusarium* sp., *Phytophthora capsici*, *Botrytis cinerea*, *Rhizoctonia solani* and *Aspergillus flavus* (Hebbar *et al*. 1998; Heydari and Misaghi 1998; Cain *et al*. 2000; Li *et al*. 2002; Vijaysamundeeswari *et al*. 2010). *Burkholderia cepacia* has been found to control *Schizophyllum commune*, the causal agent of seed rot of oil palm (Janisewiez and

Roitmann 1988; Dikin *et al*. 2003). Suparman *et al*. (2002) demonstrated that an isolate of *B.cepacia* obtained from the rhizosphere of tomato inhibited the spore germination of *Fusarium oxysporum* f.sp. *lycopersici*, the causal agent of fusarium wilt of tomato. Sijam and Dikin (2005) reported that *B.cepacia* isolate BC-S inhibited the mycelia growth of *Schizophyllum commune* and *Colletotrichum dematium* and the *B. cepacia* isolate BC-TM was effective in inhibiting the mycelial growth of *F. oxysporum* f.sp. *lycopersici*, *F. solani* and *Ganoderma boninense*. *Burkholderia cepacia* AMMDR1 has been reported as a biocontrol agent that protects pea and sweet corn seeds from *Pythium* damping-off under field conditions (Parke *et al*. 1991; King and Parke 1993). Heungens and Parke (2000) demonstrated that *B. cepacia* AMMDR1 caused zoospore lysis, prevented cyst germination, and inhibited germ tube growth of *Pythium aphanidermatum* and *Aphanomyces euteiches in vitro*. Also *B. cepacia* AMMDR1 reduced the attractiveness of pea seed exudates to *Pythium* zoospores to non-detectable levels. Hwang and Benson (2002) demonstrated that sequential application of *Burkholderia cepacia* strain 5.5B at propagation, followed by a binucleate *Rhizoctonia* isolate at transplanting, effectively controlled stem rot and root rot of poinsettia (*Euphorbia pulcherrima*) caused by *Rhizoctonia solani*. Watanabe *et al*. (2000) reported that *Burkholderia* sp. isolate 87-11 obtained from basidiospore of *Lentinus lepideus*, showed antagonistic activity against *Pythium aphanidermatum* and *Rhizoctonia solani*. Production of pyrrolnitrin (Baligh *et al*. 1999), pyoluteolin, cepabactin, volatile ammonia and siderophore (Lievens *et al*. 1989; Meyer *et al*. 1989) are the major mechanisms proposed for the disease suppressive effects of *B. cepacia*. The results of the present experiment confirms the plant growth promoting ability of *Burkholderia* sp. strain TNAU–1 by increasing the root length, shoot length and seedling vigour of mung bean when the seeds were bacterized with *Burkholderia* sp. Besides, the endophytic movement of *Burkholderia* sp. strain TNAU-1 in mung bean has been demonstrated in the present study. This proves to be a promising feature since the endophytic movement from the point of application to the internal tissues provide a relatively uniform and protected environment and by colonizing the internal tissues, can exclude the entry of a pathogen. It has been demonstrated that application of endophytic bacteria by stem injection in cotton plants reduced root rot caused by *Rhizoctonia solani* and vascular wilt caused *by Fusarium oxysporum* f.sp.*vasinfectum* (Chen *et al*. 1995). The results of the present study demonstrated the usefulness of the talc-based powder formulation of *Burkholderia* sp. strain TNAU-1 in the control of root rot of mung bean under greenhouse conditions. This strain warrants further investigation for its ability to control root rot of mung bean in commercial situations.

REFERENCES

Baki A.A.A., Anderson J.D. 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci. 31: 630–633.

Baligh M., Delgado M.A., Conway K.E. 1999. Evaluation of *Burkholderia cepacia* strains: Root colonization of *Catharanthus roseus* and *in vitro* inhibition of selected soil-borne fungal pathogens. Proc. Oklahama Acad. Sci. 79: 19–27.

Cain C.C., Henry A.T., Waldo R.H., Casida L.J., Falkinham J.O. 2000. Identification and characteristics of a novel *Burkholderia* strain with broad-spectrum antimicrobial activity. Appl. Environ. Microbiol. 66: 4139–4141.

Chen C., Bauske E.M., Musson G., Rodriguez-Kabana R., Kloepper J.W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Control 5: 83–91.

Dennis C., Webster J. 1971. Antagonistic properties of species groups of *Trichoderma* 1. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57: 25–39.

Dhingra O.B., Sinclair J.B. 1978. Biology and Pathology of *Macrophomina phaseolina*. Universidade Federal de Viscosa, Brazil, 166 pp.

Dikin A., Sijam K., Abidin M.A.Z., Idris A.S. 2003. Biological control of seedborne pathogen of oil palm, *Schizophyllum commune* Fr. with antagonistic bacteria. Int. J. Agric. Biol. 5: 507–512.

Gomez K.A., Gomez A.A. 1984. Statistical Procedure for Agricultural Research. Wiley, New York, NY, USA, 680 pp.

Hebbar K.P., Martel M.H., Heulin T. 1998. Suppression of pre and post-emergence damping-off in corn by *Burkholderia cepacia*. Eur. J. Plant Pathol. 104: 29–36.

Heungens K., Parke J.L. 2000. Zoospore homing and infection events: Effects of the biocontrol bacteria *Burkholderia cepacia* AMMDR1 on two oomycete pathogen of pea (*Pisum sativum* L.). Appl. Environ. Microbiol. 66: 192–200.

Heydari A., Misaghi I.J. 1998. Biocontrol activity of *Burkholderia cepacia* against *Rhizoctonia solani* in herbicide-treated soils. Plant Soil 202: 109–116.

Hwang J., Benson D.M. 2002. Biocontrol of Rhizoctonia stem and root rot of poinsettia with *Burkholderia cepacia* and binucleate *Rhizoctonia*. Plant Dis. 86: 47–53.

Indira N., Gayatri S. 2003. Management of blackgram root rot caused by *Macrophomina phaseolina* by antagonistic microorganisms. Madras Agric. J. 90: 490–494.

International Seed Testing Association. 1996. International Rules for Seed Testing. Seed Sci. Technol Supp. 24: 155–202.

Janisewiez W.J., Roitmann J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78, p. 1697.

Karthikeyan M., Bhaskaran R., Radhika K., Mathiyazhahan S., Jayakumar V., Sandosskumar R., Velazhahan R. 2005. Endophytic *Pseudomonas fluorescens* Endo2 and Endo35 induce resistance in black gram [*Vigna mungo* (L.) Hepper] to the pathogen *Macrophomina phaseolina*. J. Plant Interact. 1: 135–143.

King E.B., Parke J.L. 1993. Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis. 77: 1185–1188.

Li W., Roberts D.P., Dery P.D., Meyer S.L.F., Lohrke S., Lumsden R.D., Hebbar K.P. 2002. Broad spectrum antibiotic activity and disease suppression by the potential biocontrol agent *Burkholderia ambifaria* BC-F. Crop Protect. 21: 129–135.

Lievens K.H., Rijsbergen V.R., Leyns F.R., Lambert B.J., Tenning P., Swings J., Joos J.P. 1989. Dominant rhizosphere bacteria as a source of antifungal agents. Pestic. Sci. 27: 141–154.

Meyer J.M., Hohnadel D., Halle F. 1989. Cepabactin from *Pseudomonas cepacia*, a new type of siderophores. J. Gen. Microbiol. 135: 1479–1487.

Muthomi J.W., Otieno P.E., Cheminingwa G.N., Nderitu J.H., Wagachaja J.M. 2007. Effect of legume root rot pathogens and fungicide seed treatment on nodulation and biomass accumulation. J. Biol. Sci. 7: 1163–1170.

Parke J.L., Rand R.E., Joy A.E., King E.B. 1991. Biological control of Pythium damping-off and Aphanomyces root rot of peas by application of *Pseudomonas cepacia* or *Pseudomonas fluorescens* to seed. Plant Dis. 75: 987–992.

Raguchander T., Samiyappan R., Arjunan G. 1993. Biocontrol of *Macrophomina* root rot of mungbean. Indian Phytopathol. 46: 379–382.

Rajendran L., Samiyappan R. 2008. Endophytic *Bacillus* species confer increased resistance in cotton against damping off disease caused by *Rhizoctonia solani*. Plant Pathol. 7: 1–12.

Rettinassababady C., Ramadoss N., Thirumeni S. 2002. Management of root rot [*Macrophomina phaseolina* (Tassi.) Goid] of rice fallow blackgram by resident isolates of *Trichoderma viride*. Indian J. Agric. Res. 36: 118–122.

Riker A. J., Riker R. S. 1936. Introduction to Research on Plant Disease. John S. Swift Co., INC., New York, 117 pp.

Saravanakumar D., Harish S., Loganathan M., Vivekananthan R., Rajendran L., Raguchander T., Samiyappan R. 2007. Rhizobacterial formulation for the effective management of *Macrophomina* root rot in mungbean. Arch. Phytopathol. Plant Protect. 40: 323–337.

Sijam K., Dikin A. 2005. Biochemical and physiological characterization of *Burkholderia cepacia* as biological control agent. Int. J. Agri. Biol. 7: 385–388.

Su G., Suh S.O., Schneider R.W., Rusin J.S. 2001. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology 92: 120–126.

Suparman M., Kamaruzaman S., Sariah M., Inon S. 2002. *In vitro* Screening of Antagonistic Bacteria against *Fusarium oxysporum* f.sp. *lycopersici*. Programme and abstracts 25th Malaysian Microbiology Symposium and 5th UNESCO National Workshop for the Promotion of Microbiology in Malaysia, 24 pp.

Taylor S.R., Weaver B.D., Wood W.C., van Edzard S. 2005. Nitrogen application increases yield and early dry matter accumulation in late-planted soybean crop. Sci. J. 45: 854–858.

Thilagavathi R., Saravanakumar D., Ragupathi N., Samiyappan R. 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. Phytopathol. Mediterr. 46: 157–167.

Thompson D.C. 1996. Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant Dis. 80: 856–862.

Vijayasamundeeswari A., Vijayanandraj S., Paranidharan V., Samiyappan R., Velazhahan R. 2010. Integrated management of aflatoxin B_1 contamination of groundnut (*Arachis hypogaea* L.) with *Burkholderia* sp. and zimmu (*Allium sativum* L. x *Allium cepa* L.) intercropping. J. Plant Interact. 5: 59–68.

Watanabe T., Tsukamoto T., Shirata A. 2000. Potential of antagonistic isolate obtained from *Lentinus lepideus* basidiospores as a biocontrol agent. Mycoscience 41:79–82.

Wyllie T.D. 1993. Charcoal Rot. p. 30–33. In: "Compendium of Soybean Diseases" (J.B. Sinclair, P.A. Backman, eds). 3rd ed. The APS Press. St. Paul, MN, 106 pp.

POLISH SUMMARY

SZCZEP TNAU-1 BAKTERII *BURKHOLDERIA* SP. JAKO CZYNNIK BIOLOGICZNEGO ZWALCZANIA ZGNILIZNY KORZENI FASOLI ZŁOTEJ (*VIGNA RADIATA* L.) POWODOWANEJ PRZEZ *MACROPHOMINA PHASEOLINA*

Oceniano możliwość wykorzystania szczepu TNAU-1 bakterii *Burkholderia* sp. jako czynnika biologicznego zwalczania zgnilizny korzeni fasoli złotej (V*igna radiata* L.) powodowanej przez grzyb *Macrophomina phaseolina* w warunkach szklarniowych. Szczep TNAU-1 bakterii *Burkholderia* sp. hamował *in vitro* wzrost grzybni *M. phaseolina* i wytwarzał strefę inhibicji równą 18,8 mm. Siewki roślin fasoli wyrastające z nasion potraktowanych zawiesiną bakterii wykazały istotny wzrost długości korzeni i łodyg, a także zwiększony wigor. Opracowano preparat zawierający szczep TNAU-1 bakterii *Burkholderia* sp. w formie proszku, na bazie talku, do zwalczania zgnilizny korzeni w warunkach szklarniowych. Preparat zastosowany bezpośrednio na nasiona lub doglebowo istotnie ograniczał nasilenie występowania choroby i wpłynął na procent kiełkujących nasion i wysokość roślin. Samo zaprawianie nasion przy pomocy preparatu w formie proszku było skuteczne przeciwko zgniliźnie korzeni, jednak zaprawianie nasion połączone z doglebowym stosowaniem preparatu zwiększało jego skuteczność. Zaprawianie nasion łącznie z zastosowaniem bakterii *Burkholderia* sp. spowodowało ograniczenie występowania zgnilizny korzeni z 52,6% (nasiona nie traktowane bakteriami) do 16,7%. Skuteczność zwalczania zgnilizny korzeni przy wykorzystaniu bakterii *Burkholderia* sp. nie różniła się istotnie od działania preparatu karbendazym, zastosowanego do zaprawiania nasion. Przemieszczanie się endofitycznych bakterii *Burkholderia* sp. w korzeniach, łodydze i liściach potwierdzono przy pomocy metody PCR, wykorzystując specyficzne startery dla bakterii *Burkholderia* sp., dające amplifikację produktu wielkości 417 pz.