# Biological control of bean root rot disease caused by *Rhizoctonia* solani under green house and field conditions

### Ahed a. H. Matloob\* and kamil s. Juber

### Department of Plant Protection, College of Agricultural, University of Baghdad-Iraq ABSTRACT:

This study was carried out to evaluate the efficiency of the biocontrol agents Trichoderma harzianium, Glomus intraradices and Azotobacter chroococcum to protect bean, Phaseolus vulgaris L. plants against Rhizoctonia solani, causative agent of root rot under different conditions. Results obtained showed that the disease was distributed in all bean cultivated area of Babylon city-Iraq and R. solani was detected in most tested samples. It has been found that R. solani had effects on bean seed germination and plant growth. Result of differential hosts revealed that R. solani isolates obtained belong to anastomosis groups 4 (AG-4) and this confirmed by the result of the dual culture test on PSA medium. Four isolates of Azotobacter chroococcum, isolated from wheat, bean and sesban, Sesbania sesban L. showed high antagonistic activity against the higher pathogenic isolate of R. solani (RS-3) on PSA medium. Arbuscular mycorrhizal, G. intraradices significantly reduced the disease incidence and severity in bean plants, The combination A. chroococcum with G. intraradices found to be more effective, than each of them separately, in decreasing disease incidence and severity under green house and field conditions. T. harzianium exhibited high activity in controlling the disease and increasing vield. All biocontrol agents used in this study improved plant growth and increased yield. The biocontrol agents may play an essential role in management of root rot diseases.

Key words: Biological control, Trichoderma harzianium, Glomus intraradices, Azotobacter chroococcum and Rhizoctonia sola

#### INTRODUCTION

Rhizoctonia solani Kühn [teleomorph: Thanatephorus cucumeris (Frank) Donk.] isolates were divided to 14 anastomosis groups (AG1-AG13) and AG-bridging isolate (AG-BI), according to anastomosis reactions between them (Ogoshi, 1976; Sneh et al., 1996; Carling et al., 1988 and 2002). R. solani is a soilborne pathogen can attacks wide range of plants species such as bean causing seed decay and damping off, hypocotyl rot, root rot and web blight (Ogoshi, 1976; Abawi et al. 1985). Trichoderma species have reported to be as potent biocontrol agent against various phytopathogenic soil fungi including R. solani and Pythium spp. (Harman, 1996; Harmann et al., 2004; Howell, 2006). The efficiency of T. harzianium to inhibit fungal growth may be through competition for space and nutrients, mycoparasitism and production of antibiotic compounds, it has found that the hyphae of T. harzianium coil around the hyphae of the pathogen and penetrate the host mycelium through degrading cell wall by secretion hydrolytic enzymes followed by assimilation of cell contents (Harmann et al., 2004; Howell, 2006, Siameto et al., 2011). Mycorrhizae has

bean found to improve plant growth through enhance the acquisition of nutrients from soil, tolerance drought as well as protect plants against pathogen. these effect may be a result of that mycorrhiza enhance beneficial microo-rganisms growth in the rhizosphere which antagonism with pathogen (Filion et al 2003; Vance, 2003). Azotobacter chroococcum, reported to excrete beneficial effects on plant growth various crops different and vield of by mechanisms(Brenner et al, 2004.; Saharan, and Nehra, 2011). It was found to produce ammonia, vitamins and some growth regulators include indole acetic acid, gibberllins, and cytokines which enhance seed germination and promote plant growth (Mrkovacki and Milic, 2001; Karthikeyan and Sakthivel. 2011.). Azotobacter can also compete with pathogen for iron through production siderophores (Neilands, 1993; Hillel, 2005). The objective of this study was to evaluate the activity of Trichoderma harzianium, Glomus intrarad-ices and Azotobacter chroococcum as a bicontrol agents against Rhizoctonia solani the causal agent of root rot

disease on bean under green house and field conditions.

### MATERIALS AND METHODS

Isolation and Identification of Rhizoctonia solani: R. solani was isolated from Bean plants shown necrotic lesions on root and hypocotyls, collected from fields in nine locations in Babylon area, Iraq during 2010-2011 growing season. Small pieces of infected root and hypocotyls were surface sterilized with 0.5% sodium hypochlorite for 1 minute, rinsed with sterile distilled water and plotted dry. The pieces were placed on Potato sucrose agar (PSA) medium containing 50 mg/L of streptomycin sulfate in Petri plates of 9cm diam. The plates were maintained at 25°C for 48-72 hrs. Fungal hyphae from the margin of developing colonies were transferred into PSA. R. solani isolates were identified on the basis of hyphal characteristics. nuclear condition. hvphal anastomosis groups using differential hosts and daul cultures of isolates on PSA medium (Parmeter and Whitney, 1970; Tsuboki, et al., 1978; Herr, 1979; Carling et al., 1988; Sneh et al., 1996).

**Pathogenicity of** *Rhizoctonia solani* isolates: sterilized soil was distributed in pots of 12.5cm in diameter at 1 kg/pot. *R. solani* grown on millet seeds were added into potting soil at 1% w:w. and sown with bean seeds (10 seeds/pot). Seeds were sown in uncontaminated soil serve as control. The pots were watering and maintained in growth room at 25°C (12 hrs. photoperiod) in a complete randomized design with four replicates. The percentage of seed germination was estimated after four days of seedlings emergence. The higher pathogenic isolate was used in the next experiments.

# Evaluation of biocontrol agents activity against *Rhizoctonia solani*

**Azotobacter :** A. chroococcum were isolated from wheat, bean, and sesban Sesbania sesban L. plants rhizosphere soil on Sucrose Mineral Salt medium (SMS) medium A. chroococcum isolates were identifed by cultural, microscopic and biochemical characteristics (Brenner *et al.*, 2004). Antifungal activity of the isolates was carried out on PSA medium according to the procedure described by Fatima *et al.* (2009).

*Trichoderma* spp.: *Trichoderma* spp. were isolated from soil of bean field at Babylon province on PSA medium. Antagonistic activity of with other soil fungi.

*T. harzianum* was selected because of its known antagonistic activity of the *Trichoderma* isolate against *R. solani* which was determined by dual culture on PSA (Data not shown)

### Glomus intraradices

The Arbuscular mycorrhizal (AM) *G. intraradices* was isolated from the soil by wet sieving and decanting method (Gerdmann, and Nicolson, 1963; Schenck, and Smith, 1982). *G. intraradices* isolates were grown and maintained on bean, barley, corn and millet roots under green house conditions. *G. intraradices* inoculum (soil containing spores and hyphae) was prepared and using against *R. solani* in the next experiments.

Green house experiment: Pots (12.5 cm in diam.) containing sterile soil (1kg /pot) were sown with bean seeds (5 seed / pot). The biocontrol agents inoculum, Mycorrhizae 10g / pot, Azoto-bacter 10 ml of 4\*10<sup>8</sup> CFU/ml culture /pot and Trichoderma at 1% (w/w) from 14 days old culture on wheat bran were added into potting soil 5 days before sowing. R. solani inoculum was added to the pots at 1%(w:w). pots were distributed in green house condition in compete randomized design with 4 replicates as following; RS-3 (T1), RS-3+AM(T2), RS-3+Th(T3), RS-3+A(T4), RS-3+AM+A(T5), control (non - treated, non contaminated soil) (T6), A alone (T7), AM alone (T8), AM+A (T9) and Th alone (T10) (Arbuscular T. harzianium (Th), mycorrhizae (AM), Α. chroococcum (A) Pathogen (RS-3)), The plants carefully removed, two months of sowing and maintained for root rot severity according to the following scale, 0 = healthy roots: 1 = secondary roots are rotten; 2 = secondary root and part of taproot is rotten; 3 = taproot is rotten 4 = taproot and crown are rotten and 5=death of plant. The disease severity was calculated according to Mckinney formula (Mckinney, 1923) fresh and dry weight of plants were determined.

### Field experiments:

An experiment was carried out in the field of Plant protection department / college of Agriculture, university of Baghdad. The experiment design was field trial was conducted in a complete randomized block with 11 treatments. and 3 replicates. The treatments were the same as described in greenhouse experiment. Bean seeds were sown in rows of 1 m each, spacing of 70 cm and 25 cm between plants. *Trichoderma harzianium* grown on wheat bran and *G. intraradices* were added into the soil at 20g / plant, 5 days before sowing, while *A. chroococcum* at 20 ml of broth culture at 4\*10<sup>8</sup> CFU/ml / plant during sowing. At maturity (after 140 days of sowing) 4 plants of each row were carefully removed for determination of disease incidence and severity, plant height, fresh and dry weight, root size and yield.

## RESULTS AND DISSCAUTIONS

### Disease survey:

Root rot disease was found to be distributed in all bean fields submitted to the study with incidence ranging from 40 to 100% and severity were 18 to 75% (table 1). The highest disease incidence (100%) was observed in Mahaweel, Al- Tahria, Al-Askanderia and Al-Emam fields. Associated with highest disease severity 75, 70.3,63.8 and 56.3% respectively. The high incidence and severity of bean root rot could result from the continuous cultivation with the same crop which lead to accumulation of *R. solani* inoculum and development of more aggressive strains (Tsuboki, *et al*, 1978; Jensen *et al*, 2010).

### Isolasion and identification of Rhizoctonia solani

**isolates**: *R. solani* has been detected in almost samples collected from bean fields at different location of Babylon area (7.7-100%) (table 1). The microscopic observa-tions of new growing hyphae of the fungus revealed multinucleate cells (3-9 nucleus / cell) figure 1. the hyphal reaction between the isolates on PSA medium revealed that RS-1,RS-2, RS-3 and RS-4 isolates belonged to anastomosis group -4 (AG4). These isolates were failed to infect,

cauliflower, wheat, rice, corn and carrot (Table.2). previous studies reported that AG-4 is the main group attacking bean plants worldwide (Karaca *et al.*, 2002; Eken and Demirci, 2004).

### Pathogenicity of Rhizoctonia solani isolates

Results of pathogenicity test showed that all *R. solani* isolates were highly pathogenic to bean plants, (Table 3). RS-3 and RS-7 inhibited seed germination completely, while RS-2 and RS-4.3 reduced the germination to 10 and 27.5% respectively within 8 days of sowing compared with seed germination of 87.5% in control plants.

### Antagonistic activity of Azotobacter against

Rhizoctonia solani on cultural media: Four isolates of A. chroococcum (A1-A4) were isolated from rhizosphere soil of selected plants. All the isolates obtained found to exert an inhibition activity against solani on PSA medium (66.33-100%) R. demonstrated by dual culture method. It was reported in previous study that A. chroococcum exhibited antifungal activity against pathogenic fungi (Fatima et al., 2009; Mali and Bodhankar, 2009). The more effective isolate A2 of A. chroococcum against R. solani on the cultural medium was used in next experiments. The results obtained in the present investigation suggests that the A1,A2,A3 and A4 isolates exhibited more potential for the production of antifungal metabolites. Therefore, these could be successfully exploited to control plant pathogenic fungi in root region.

District	Field area (2500m <sup>2</sup> )	Disease incidence (%)	Severity (%)	Ratio of <i>R</i> . solani
Al-Azawia	2	80	38	25.0
Al- Tahria	2	100	70.3	9.6
Moalha	4	50	26.7	7.7
Al-Mansori	3	40	18	100
Haswa/Al-Askanderia	4	100	63.8	0.0
Mahaweel	3	100	75	0.0
AI-Emam	5	100	56.3	11.3
Mashrooa Al-Musaib	2	90	58	0.0
AI –Badaa	2	100	62.8	15.0

Table 1. Survey bean root rot disease, Rhazoctonia solani, in some fields in Babylon city during 2009 - 2010

olates	rape	radish	cabbage	cotton	bean	cowpea	onion	spinach	cauliflower	Rice	wheat	corn	carrot	potato	pepper	Egg plant	cucumber	Water melon	Cantaloupe
AG-1	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
AG-2	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
AG-3	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
AG-4	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+

Table .2 Determination the anastomosis groups of some Rhizoctonia solani isolates using differentially plants.

RS= Rhizoctonia solani, += infected , - = uninfected



Fig 1. Rhizoctonia solani, A. Anastomosis Group, perfect fusion of two hyphae of isolates( X100), B. Multinucleate of cells(X40) ٦

Table 3. Effect of	Rhizoctonia	solani isolates	on bean se	ed germination
--------------------	-------------	-----------------	------------	----------------

Isolates	RS-3	RS-7	RS-2	RS-4,3	Control ( without pathoge
Germination rate	0.0	0.0	10	27.5	87.5
DS_ Dhizaatania aalani	the number bee	ida tha igalata	represent inclote	number 1	$\frac{1}{2}$ D (p 0 0 5) - 1(

RS= Rhizoctonia solani, the number beside the isolate represent isolate number, L.S.D. (p=0.05)=10.6

### Table. 4 Growth inhibition of Rhizoctonia solani (RS-3) by A. chroococcum in PSA medium

Treatment	Growth diameter/Cm2	Inhibition (%)
RS-3+A1	0.00	100.00
RS-3+A2	0.00	100.00
RS-3+A3	0.00	100.00
RS-3+A4	3.03	66.33
RS-3	9.00	0.00

RS= Rhizoctonia solani, A= Azotobacter chroococcum, the number inside the letter is number of isolate, (A1(Baghdad,wheat), and A2, A3, A4 (Babylon, bean, wheat, seasban)). L.S.D. (p=0.05)=1.44

Effeciency of biocontrol agents on disease incidence and severity under green house conditions: It has been shown (Table 5) that all the bioagents used in this study induced significant reduction in both incidence and severity of bean root rot, caused by R. solani under green house conditions. Mycorrhizal colonization of bean root reduced root rot incidence and severity to 43.8% and 23%, Azotobacter chroococcum to 37.5% and 20.0% and to 25% and 15% in combination of Azotobacter and Mycorrhizae, compared with 100% and 80% in soil contaminated with RS-3 isolate respectively. T. harzianum was found effective in reduction of disease incidence (31.2%) and disease severity 18.8%. Beltanol showed more reduction effects of disease incidence 12.5% and severity 7.5%. In addition to antifungal effects against R. solani, bean root rot. The biocontrol agents were found to improve plant growth as shown by the significant increases in fresh and dry weights of bean plants compare with control (R. solani alone). R. solani is a soil-borne pathogen which attacks a wide range of plants (Ogoshi, 1976). In bean, Phaseolus vulgaris L., R. solani can cause several types of damage, including hypocotyl rot, seed and root rot and web blight (Abawi et al., 1985). There have been many reports on the beneficial effects of A. chroococcum on growth and yield of various agriculturally important crops, It benefits plants in many ways, includes the produce ammonia, vitamins and growth substances. that enhance seed germination, production of indole acetic acid and other auxins such as gibberllins and cytokinins, nutrient absorption, inhibition of phytopathogenic fungi through antifungal substances and production of siderophores which solubilize Fe3+ and suppress plant pathogens through iron deprivation (Neilands, 1993; Mrkovacki and Milic 2001; Vaddar, 2007). Whereas, T. harzianium mechanisms includes competition for space and nutrients (Harman, 1996), mycoparasitism (Harmann et al. 2004), production of antifungal agents and hydrolytic enzymes, inactivation of the pathogen's enzymes, induce resistance and enhancement of plant growth (Howell, 2006).

Table 5.	Influence	of	biocontrol	agents	on	incidence	and	severity	of	bean	root	rot	disease	under	green	house
								conditi	one	•						

		condition	3	
treatment	Disease incidence	Severity (%)	fresh weight (g)	Dry weight (g)
RS-3	100.0	80.0	1.36	0.162
RS-3+A2	37.5	20.0	2.81	0.308
RS-3+Gi	43.8	23.8	2.68	0.303
RS-3+A2+Gi	25.0	15.0	3.22	0.320
RS-3+Th	31.2	18.8	3.25	0.305
RS-3+Bel	12.5	7.5	3.62	0.360
Control	0.0	0.0	3.97	0.380
A2	0.0	0.0	4.42	0.435
Gi	0.0	0.0	4.22	0.410
A2+Gi	0.0	0.0	4.55	0.460
Th	0.0	0.0	4.37	0.430
L.S.D.( <i>P</i> = 0.05)	11.7	5.4	0.22	0.024

RS-3=*Rhizoctonia solani* isolate 3, A2 =*Azotobacter chroococcum* isolated from bean rhizosphere, Gi=*Glomus intraradices* Th=*Trichoderma harzianium*, Bel = Beltanol fungicide, Control= without pathogen

### **Field-experiment:**

Results showed that all biocontrol agents used in this study induced significant reduction in root rot incidence and severity as well as improved plant growth and yield compared with control treatment (RS-3 alone) (Table 6). The combination of *A. chroococcum* with *G. intraradices* was found to be more effective, the disease incidence, disease severity, plant height, root size, fresh weight, dry weight and yield in this treatment were found 33.3%, 21.6%, 57.7cm, 6.7cm, 2.03g, 512.3g and 1.95kg respectively, compared with 100%, 76.8%, 31.3cm, 3.0cm, 0.63g, 213.7g and 0.61kg in control (RS-3 alone) treatment respectively.

The results of this study demonstrated that bean root rot is distributed wherever bean is cultivated in Babylon area-Iraq. The use of A. chroococcum, G. intraradices and T. harzianium as biocontrol agents to manage the disease were found very effective in reducing root rot incidence and severity caused by the more aggressive isolate of R. solani (RS-3) as well as improved plant growth of bean plants. The improvement of plant growth could result from that biocontrol agents produced during their growth on and around plant roots, substances acts as growth regulators as well as make certain nutrients more available into uptake by roots lead to stimulate plant growth (Mrkovacki and Milic. 2001; Harmann et al, 2004; Rajeshkumar et al., 2009; Karthikeyan and Sakthivel 2011; Saharan, and Nehra. 2011). It is possible also that the improvement of plant growth may be due to restriction R. solani growth and suppression of disease incidence. The activity of biocontrol agents against R. solani may be directly through competition on nutrients, siderophore mediated competition for iron. antibiosis, or through secretion lytic enzymes (Harman, 1996; Ordookhani and Zare, 2011). The effects of beltanol was referred to as combined with copper mineral which transfer in plant tissues and killed the pathogens (Meister, 2000). These results were in accordance with those obtained by Dar et al (1997) how found that inoculation of common bean plants with Glomus mosseae decreased bean root rot by 34 to 77%. Many researchers have reported that the AM colonization can reduce root rot disease caused

by several soil borne fungi (Filion et al, 2003; AL-Askar and Rashad). Among the potential mechanisms involved in the resistance of mycorrhizal systems is the induction of plant defense (Jung et al., 2009). Results also were in agreement with EL-Barugy et al (2009) who found that A. chroococcum have high antagonistic ability against root rot pathogens under green house and field conditions and increased some plant growth parametres. and with Al-Askar and Rashad (2010) which reported that roots of bean plant which were colonized by G. intraradices were resistance to Fusarium root rot and increased growth of plants and its nutrients contents, phenolic compounds and resistance related enzymes.

Farrag (2011) found that R.solani was very aggressive on different bean varieties and caused 94% disease incidence in susceptible varieties and 39% in resistant varieties and caused reduction in length and weight of roots. This pathogen is one of soil-borne fungi which attack seeds, seedlings, and roots of beans, causing seed rot and pre or post emergence damping-off of seedlings, root rot , stem necrosis, yellow and weak plants and decrease quantity and quality of yield (Abawi et al, 1985). Many researches showed the effective integrated Azotobacter addition of and Arbuscular mycorrhizae in improving plant growth (Rajeshkumar et al., 2009; Bahrani et al., 2010; Ordookhani, and Zare, 2011). The reduction of disease incidence and severity by biocontrol agents may be directly through activation plant defense mechanism and induce systemic resistance in plant against R. solani lead to produce substances in the plants including proteins acts as antifungal agents (Vance, 2003; Harmann et al., 2004; Howell, 2006; Saharan, and Nehra, 2011). It can be concluded that the biocontrol agents Α. chroococcum. G intraradices and T. harzianium decreased root rot disease incidence and increased plant resistance against infection with R. solani and improve plant growth and yield. These results demonstrated that the biocontrol used in this study may be promising in management of root rot diseases

treatment	Disease incidence	Severity (%)	High of plant	Root size (cm3)	Vet weight (g)	Dry veight (g)	Yield (Kg)
			(cm)				
RS-3	100.0	76.8	31.3	3.0	0.63	213.7	0.61
RS-3+A2	41.7	28.3	54.3	6.3	1.80	490.0	1.89
RS-3+Gi	50.0	38.3	50.0	6.0	1.63	482.7	1.72
RS-3+A2+Gi	33.3	21.6	57.7	6.7	2.03	512.3	1.95
RS-3+Th	50.0	26.8	55.3	6.5	1.83	487.3	1.83
RS-3+Bel	16.7	6.7	61.7	6.8	2.14	530.3	2.19
Control	16.7	6.7	65.0	6.9	2.53	565.3	2.30
A2	0.0	0.0	67.3	7.4	2.73	586.7	2.80
Gi	0.0	0.0	67.0	7.4	2.67	583.7	2.72
A2+Gi	0.0	0.0	68.3	7.7	2.86	613.0	2.87
Th	0.0	0.0	66.7	7.3	2.60	589.3	2.74
L.S.D.( P= 0.05)	14.7	7.8	2.43	0.21	0.14	8.50	0.063

Table 6. Influence of biocontrol agents on disease incidence and severity under field conditions

RS-3=Rhizoctonia solani isolate 3, A2 = Azotobacter chroococcum isolated from bean rhizosphere, Gi=Glomus intraradices Th=Trichoderma harzianium, Bel = Beltanol fungicide, Control = without pathogen each value represent mean of 4 plant in replicate and 3 replicates in treatment.

#### REFERENCES

- Abawi, G.S. D.C. Crosier and A. C. Cobb. 1985. Root rot of snap bean in New York. New Yorks food and life life Sci. Bulletin, 110:362-369
- AL-Askar, A. A. and Y. M. Rashad. 2010. Arbuscular mycorrhizal fungi: A biocontrol agent against common bean Fusarium root rot disease. Plant Pathol. J. 9:31-38.
- Bahrani, A., J. Pourreza and M. H. Joo. 2010. Response of Winter wheat to co-inoculation with *Azotobacter* and arbuscular mycorrhizal fungi (AMF) under different sources of nitrogen fertilizer. American- Eurasian J. Agric. Environ. Sci. 8:95-103.
- Brenner, D.J., N.R.Krieg and J.T. Staley. 2004. Bergey's manual of systematic bacteriology. Williams and Wilking.Baltimore. London .1136pp.
- Carling, D. E., S. Kuninaga and R. H. Leiner . 1988 . Relatedenes within and among intraspecific groups of *Rhizoctonia solani* A comparison of grouping by anastomosis and by DNA hybridization. Phytoparasitica. 16 : 209 – 210.
- Carling, D. E., R. E. Baird, R. D. Gitaitis, K. A. Brainard and S. Kuninaga. 2002. Characterization of AG-13, a newly Reported anastomosis group of *Rhizoctonia solani*. Phytopathology . 92:893-899.
- Dar, G.H.,M.Y.Zargar and G.M.Beigh. 1997. Biocontrol of Fusarium root rot in common bean ( *Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microb. Ecol.,34: 74-80.

isolates from bean in Erzurum, Turkey. J. of Plant Pathol. 86: 49-52.

- EL-Barougy, E., N. M. Awad, A. S. Turky and H. A. Hamed. 2009. Antigonistic activity of selected strains of Rhizobacteria against *Macrophomina phaseolina* of soybean plants. American- Eurasian J. Agric& Environ. Sci. 5: 337-347.
- Farrag, A. A. 2011. Efficiency of different biocontrol agents on both susceptible and resistant bean plants and their protein pattern consequences. J. of American Sci. 7 : 7-14.
- Fatima, Z., M. Saleemi, M. Zia, T. Sultan, M. Aslam, R. U. Rehman and M. F. Chaudhary. 2009. Antifungal activity of plant growth- promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. African J. of Biotech. 8: 219-225.
- Filion, M., M. St-Arnaud and S. H. Jabaji-Hare.2003. Quantification of *Fusarium solani* f.sp *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using realtime polymerase chain reaction and direct isolations on selective media. Phytopathology 93:229-235.
- Gerdmann, J. W. and T.H. Nicolson. 1963. Spore of mycorrhizal endogene species extracted from soil by wet-sieving and decanting. Trans. Brit. Mycol. Soc.,46:235-239.
- Harman , G. E. 1996 . *Trichoderma* for biocontrol of plant pathogens : from basic research to commercialized products. Cornell community , conference on biological Control, Cornell Univ. 7pp.
- Harman, G. E., C. R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* Species-Opportunic, Avirulent Plant Symbiosis. Natural Rev. Microbiol. 2:43-56.

Herr, L. J . 1979 . Practical nuclear staining procedures for *Rhizoctonia* – like fungi. Phytopathology 69: 958–961.

Eken, C. and E. Demirci. 2004. Anastomosis groups arhitel, D. 2005. Plant Growth Promoting Bacteria. Elsevier, pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* xford, U. K.,:103-115.

- Howell, C. R. 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology 96: 178-180.
- Jensen,C.E.,J.E.Kurle,andJ.A.Percich.2010.Integrated strategies to control bean root rot in Minnesota. Northarvest bean growers association.
- Jung, S. C., J. Garcia- Andrade, A. Verhage, I. M. Fernandez, J. M. Garcia, C. Azcon- Aguilar and M. J. Pozo. 2009. Arbuscular mycorrhiza confers systemic resistance against gray mold (*Botrytis cinerea*) in tomato through priming of JA- dependent defense responses. 5<sup>th</sup> meeting of the IOBC Working Group, Granada, Spain, 12-16 May 2009.
- Karaca, G. H., I. Ozkoc, and I. Erper. 2002. Determination of the anastomosis grouping and virulence of *Rhizoctonia solani* Kuhn isolates associated with bean plants growth in Sumsun / Turkey. Pak. J. of Biol. Sci. 5 : 434-437.
- Karthikeyan, A. and K. M. Sakthivel. 2011. Efficacy of *Azotobacter chroococcum* in rooting and growth of *Eucalyptus camaldulensis* stem cuttings. Res. J. of Microbiol. 1-7.
- Mali, G. V. and M. G. Bodhankar. 2009. Antifungal and phytohormone production potential of *Azotobacter chroococcum* isolates from groundnut (*Arachis hypogea* L.). Asian J. Exp. Sci. 23: 293-297.
- Mckinney, H.H..1923.Biological control of nematode pests by natural enemies. Ann. Rev. Pytopathol. 18:415-440.
- Meister, R. T. 2000. Farm chemical handbook. Listing for "Beltanol ". Willouhg by OH. 86 : 45p.
- Mrkovacki, N. and V. Milic. 2001. Use of *Azotobacter chroococcum* as potentially useful in agricultural application. Annals of Microbiol.51: 145-158.
- Neilands, J. B. 1993. Prespectives in biochemistry & biophysics sidero-phores. Archives of Biochem. Biophys. 302:1-3.
- Ogoshi, A.1976. Studies on the grouping of *Rhizoctonia solani* Kuhn with hyphal anastomosis, and on the perfect stage of groups.Bull.Natl. Inst.Agric. Sci. Ser.C.30:1-63.
- Ordookhani, K. and M. Zare. 2011. Effect of *Pseudomonas, Azotobacter* and arbuscular mycorrhiza fungi on lycopene, antioxidant activity and

total soluble in tomato (*Lycopersicon esculentum* F1 Hybrid, Delba). Advances in Environ. Biol. 5: 1290-1294.

- Parmeter, J. R. and H. S. Whitney. 1970. Taxonomy and nomencleature of the imperfect stage In: *Rhizoctonia solani* Biology and pathology. Parmeter, J. R. Univ. of California . 7–19.
- Rajeshkumar, S., M. C. Nisha, P. C. Prabu, L., Wondimu and T. selvaraj. 2009. Interaction between *Glomus geosporum, Azotobacter chroococcum* and *Bacillus coagulans* and their influence on growth and nutrition of *Melia azedarach* L., Turk J. Biol. 33:109-114.
- Saharan, B.S. and V. Nehra. 2011. Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medicine Research, LSMR-21,30 pp.
- Siameto, E. N., S. Okoth, N. O. Amugune, and N. C. Chege. 2011. Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from Embu District, Kenya. Tropical and Subtropical Agroecosystems. 13:81-90.
- Schenck,N.C.andG.S.Smith.1982.Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida, Mycologia,74-77. In: Powell, C. L. and D. J. Bagyaraj. 1984. VA Mycorrhiza. CRC Press, Inc. Boca Raton, Florida. 234pp.
- Sneh, B., S. Jabaji- Hare, S. Neate and G. Dijst. 1996. *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, London. 578pp.
- Tsuboki, K., H. Abe and T. Aota . 1978. Black scurf of potato with malformed tubers caused by *Rhizoctonia solani* in abalshiri district of Hokkaido. Bull. Hokkaido Exp. St. 37: 68 77.
- Vadakattu, G. and J. Paterson . 2005 . *Rhizoctonia* a disease menace for many crops . Farming Ahead . 157 : 51–56.
- Vaddar, U. B. 2007. Studies on grape rhizosphere microorganisms. Master Thesis. Univ. of Agric. Sci. Dharwad. 91 pp.
- Vance, C. P. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing anonrenewable resource. New phytologist. 157:423-447