EUROPEAN ACADEMIC RESEARCH
Vol. II, Issue 12/ March 2015

Impact Factor: 3.1 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Effect of carbendazim and solarized soil with *Pseudomonas fluorescens*, spent mushroom compost against *Fusarium oxysporum* f.sp. *lycopersici* in Tomato

HUSSEIN ALI SALIM
SOBITA SIMON
Department of Plant Pathology & Entomology
Sam Higginbottom Institute of Agriculture, Technology & Sciences
(Deemed University), Allahabad, India

Abstract:

This study was planned to assess the efficacy of Pseudomonas fluorescens, spent mushroom compost and carbendazim with and without soil solarization against Fusarium oxysporum f.sp. lycopersici that cause wilt of tomato in pots condition in the green house . A total of seven treatments, were taken up in randomized block design. Sick soil of Fusarium oxysporum f.sp. lycopersici was made @ cfu 3× 10^5/ g before solarization. After solarization FYM was applied@ 100 g / pot of each treatment. P .fluorescens @2g, spent mushroom compost @ 20 g and carbendazim @ 2g / kg soil were applied in the pots.The susceptible tomato cultivar Co-3 were sown to each pot, seven days after germination 4 seedlings per pots were maintained. Results shows that the treatment of P .fluorescens with solarized soil and carbendazim increased plant vigour and significantly reduced Fusarium oxysporum f.sp. lycopersic disease intensity as compared to other treatments. Maximum root length was noticed in T4 (solarized soil+ Spent mushroom compost+ P .fluorescens) followed by T3(solarized soil+ P .fluorescens) and T2(solarized soil+ Spent mushroom compost). Maximum fresh and dry shoot weight also recorded in P .fluorescens + Spent mushroom compost plants as compared to P .fluorescens and Spent mushroom compost alone treated plants. Among the treatments, the reduction% of disease was recorded in P .fluorescens incombination with spent mushroom compost shows maximum reduction as compared with other treatments.

Key words: *Fusarium oxysporum* f.sp. *lycopersic*, *Pseudomonas fluorescens*, spent mushroom compost, Carbendazim, solarized soil and Tomato plant

Inroduction

Fusarial wilt caused by *Fusarium oxysporum* schlechtent.f.sp. *lycopersici* (Sacc.) Synder and Hans is a destructive disease of tomato crops worldwide (Sheu and Wang, 2006). The losses due to this disease ranging from 10 to 80 per cent have been reported from different parts of India. *Fusarium* species are present in soil both in temperate and tropical regions and are most frequently isolated.

Armstrong, (1981) reported *Fusarium oxysporum* (Schlecht) is a cosmopolitan fungus that exists in many pathogenic forms. It survives in soil in the form of chlamydospores and mycelia. The mycellium is septate, hyaline, branched and intra-cellular. Once introduced it remains at all stages of plant growth, starting from nursery upto flowering, it causes Clearing of veinlets and drooping of petioles of young plants lower leaves show yellowing and later the whole plant wilts and die prematurely. Browning of vascular system can be seen in a cross section of the lower stem. In wet weather condition fungal mycelial growth can be seen on dead plants in the form of pinkish mycelial layers (Singh, 1995)

Biological approach is eco-friendly, does not leave any residual toxicity, besides being cost effective and can be successfully exploited in the framework of integrated disease management. Application of a single antagonist often results in inconsistent management of the disease and the antagonistic strain may not grow equally well in a variety of environmental conditions. One of the strategies to overcome this problem is to combine the disease suppressive activity of two or more beneficial antagonists. Application of organic amendments to

soil is a traditional practice in Indian Agriculture. Besides providing nutrients to plants organic matter reduces the inoculums density of soil borne pathogens through changes in the general microbial balance of soil (Lukade, 1992). Several research groups have tested the efficacy of antagonistic microbes for the control of *F.oxysporum* (Madi *et al*., 1997; Tsahouridou and Thanassoulopoulos, 2002; Errakhi *et al*., 2007). which induces diseases difficult to control because of the production of sclerotia that represent resistant survival structures (Elad, 1995). *Fluorescent pseudomonads* have been reported as promising biological control agents against *F. oxysporum* in betelvine (Singh, 2003) and bean (De La Fuente *et al*., 2004). *Fluorescent pseudomonads* are nonpathogenic rhizobacteria (Saravanan *et al*., 2004; Karthikeyan *et al*., 2006) and several isolates of *Pseudomonas fluorescens*, *P. putida*, *P. aeruginosa* and *P. aureofaciens* suppressed the soil born pathogens through different proposed mechanisms including rhizosphere colonization, antibiosis and iron chelation by siderophore production (Karthikeyan *et al*., 2006). In plants, certain secondary metabolite pathways are induced by infection with microorganisms (Abdul Jaleel *et al*., 2009). Our aim of the work was to test the effect of a *Pseudomonas fluorescens* and spent mushroom compost against *F.oxysporum* f.sp. *lycopersici* disease development and plant growth of tomato.

Materials and methods

Isolation of *F. oxysporum* f. sp. *lycopersici*:

F. oxysporum f. sp. *lycopersici* was isolated from infected tomato plant roots by tissue segment method. The infected roots were cut separately into small pieces with the help of sterile blade and surface sterilized with mercuric chloride solution (0.1%) for 20-30 seconds followed by three times rising with sterilized distilled water. The pieces were then placed on sterilized blotting paper to remove excess moisture. The surface sterilized

diseased pieces were then aseptically, transferred on czapek's dox agar medium and incubated at 28±2˚C for seven days. After incubation, white colour colonies were observed and identified on the basis of morphological and reproductive characters. The pure culture was maintained on czapek's dox agar and preserved at low temperature in refrigerator followed by the method of Raithak and Gachande , (2013).

Preparation the sick microplot of *F. oxysporum* f. sp. *lycopersici*:

The soil was infest uniformly with the wilt pathogen, 1 L of a sand/ sorghum grains inoculum mix was incorporated into the top 45 cm of 6 microplots. The inoculum was prepared by growing a culture of *Fusarium oxysporum* f.sp. *lycopersici* in a modified liquid broth czapek's Dox (CD) (Esposito and Fletcher, 1961) on a rotary shaker with indirect fluorescent light at room temperature (22º C). After 15 days, the contents of the flask were filtered through eight layers of sterile cheesecloth. One hundred milliliters of the filtrate, which was predominantly microcondia, was mixed with 4 L of a twice-autoclaved sand / sorghum grains (4:1, v/v) mixture. The mixture was incubated 8 wk at room temperature to allow the fungus to colonize the medium extensively. To verity that *Fusarium oxysporum* f.sp. *lycopersici* had been established successfully, microplots were planted with seeds of the wilt-susceptible tomato cultivar CO - 3. After, a high percentage of wilt had occurred in all infested plots while no wilt occurred in uninfested two microplots. The remaining aboveground plant material was cut off at the soil line and removed. On 2nd May 2013, each microplot was light irrigated and covered with an ultraviolet stabilized, 30 µm clear polyethylene film in 4 microplots. The plastic was removed thrice from six microplots at 15 days of interval that is up to June 2013 (Martyn and Hartz, 1986), two microplots was kept in nonsolarized soil.

Application of *Pseudomonas fluorescens*, spent mushroom compost and Carbendazim:

The solarized and unsolarized soil was mixed with FYM @ 100 g / pot and was filled in thirty five pots. Out of thirty five, 25 pots were filled with solarized soil , Carbendazim 50 % w.p applied @ 2 kg a.i / ha, whereas spent Mushroom compost was mixed with soil in the pots @ 20 g / kg of soil and *Pseudomonas fluorescens* @ 2 g / pot 4 days before sowing the seeds of tomato variety (CO-3)**.** Seven days after germination, four seedlings per pot were maintained in each treatment. Three untreated pots were served as control. Observations were recorded on shoot length (cm), fresh and dry shoot weight (g), root length (cm), fresh and dry root weight (g), disease intensity and reduction (%) of *Fusarium oxysporum* f.sp. *lycopersici.* The percentage of disease intensity was determined using the formulas as given by Song *et al*. (2004).

0: No wilt symptoms

1: Slight severity, where 25% leave become wilted and one or two leaves became yellow

2: Moderate severity, two or three leaves became yellow, 50% of leaves became wilted

3: Extensive severity, the all plant leaves became yellow, 75% of leaves become wilted and growth is inhibited

4: Complete severity, the whole plant leaves become yellow, 100% of leaves become wilted and the plants die.

$$\text{Disease intensity (\%)} = \frac{\sum \text{Scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \times 100$$

Reduction (%) of disease was calculated by using the following formula according to (Hend and Kahkashan *et al*., 2012).

$$\text{Reduction (\%)} = \frac{\text{Control- treatment}}{\text{Control}} \times 100$$

Results and discussions

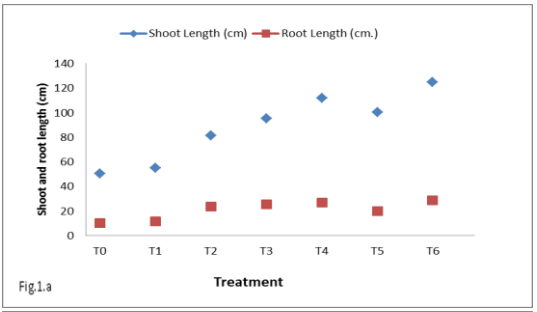
Among the different treatments tested excluding T_6 (Tomato plant alone), T_4 (solarized soil + *Pseudomonas fluorescens* + spent mushroom compost +*F o*) and T_5 (carbendazim +*F o*) increased plant vigour and consistently reduced the disease intensity under green house conditions (Table 2). The shoot length of tomato in T2 (S.S+s.m.c+ *F.o*) and T3 (S.S+ *P.f*+ *F.o*) significantly increased from T0 (non S.S+ *F.o*) and T1 (S.S. + *F.o*). Amog the treatments (T0, T1), (T2, T3) and (T4, T5) were found not significant among each other. Significantly increased in fresh shoot weight of Tomato was recorded in T4 (62.2g) followed by T5 (50.40g), T3 (37.20) from T2 (40.70). Dry shoot weight of tomato was found not significant in T2 (21.95), T3 (22.8), T4 (36.4) and T5 (30.45). The root length of Tomato significantly increased in T4 (26.95) and T3 (25.70) as compared with T5 (20.00). Whereas (T0, T1) ;(T2, T5) and (T4, T3) were found not significant among each other. The fresh root weight inT5 (4.40g), T2 (4.35g) and T4 (3.80g) was significantly increased as compared with T1 (0.79g) and T0 (0.60g). However (T5, T2, T4) and (T1, T0) were found not significant from each other. The dry root weight of tomato in all the treatments (T2, T5, T4, T3, T1, T0) were found not significant from each other excluding T6 (Plant alone). At 60 days the percentage disease intensity was significantly reduced in T_5 (6.25%) followed by T4 (7.50%), T3 (8. 75%) and T2 (10.00%) as compare to T1 (20.0%) and T0 (23.7%). However the treatments (T0, T1) (T2, T3, T4, T5) and (T1, T2, T3) are not significant from each other. The reduction percentage over control are in order T1 (15.7) smaller than T2 (57.8) smaller than T3 (63.1) smaller than T4 (68.4) smaller than T5 (73.6) .At 90 days percentage significantly

reduced in T5 (12.4), T4 (15.0), T3 (16.2) and T2 (21.2%) as compared toT0 (36.2%), the treatments (T2, T3, T4, T5) are not significant each other, the reduction percentage is maximum in T5 followed by T4, T3, T2 and T1 as compared to T0. At 120 and 150 days of germination the treatments significantly reduced the disease intensity in T5 (16.2%) and (17.2%) as compared with T0 (49.9%) and (65.3%) respectively , the treatments (T5, T4, T3 and T2) were found not significant from each other as compared with T0 and T1. disease intensity reduction % *F. oxysporum* f. sp. *lycopersici* was significantly reduced in T_5, T4, T_3, and T2 at 120 days of germination from T_1 (30.8 %) ,T_0 (49.9 %) and at 150 days of germination from T_1 (41.4 %) ,T_0 (65.3 %).

Table 1: Effect of Carbendazim and solarized soil with *Pseudomonas fluorenscens*, spent mushroom compost on plant growth parameters of tomato plants at 150 days after treatment.

Treatment		Shoot length (cm)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	Root Length (cm.)	Fresh Root Weight (gm)	Dry Root Weight (gm)
T0	*F. o* alone	50.65	7.50	5.05	10.35	0.60	0.39
T1	S.S + *F. o*	55.10	8.650	5.60	11.55	0.79	0.52
T2	S.S + s.m.c +*F. o*	81.25	40.70	21.95	23.85	4.35	1.90
T3	S.S + *P.f* +*F. o*	95.50	37.20	22.80	25.70	2.60	1.10
T4	S.S + s.m.c + *P.f* +*F. o*	112.00	62.20	36.40	26.95	3.80	1.40
T5	C +*F. o*	100.55	50.40	30.45	20.00	4.40	1.85
T6	Tomato plant alone	124.81	91.35	61.60	28.65	6.20	3.30
F- test		S	**S**	**S**	**S**	**S**	**S**
S. Ed. (±)		7.344	12.861	8.531	2.152	0.516	1.217
C. D.(P =0.05)		15.569	27.265	18.087	4.562	1.094	2.581

Solarized soil = S.S
Fusarium oxysporum = *F.o*
spent mushroom compost = s.m.c
Carbendazim = C
Pseudomonas fluorescens=*P. f*



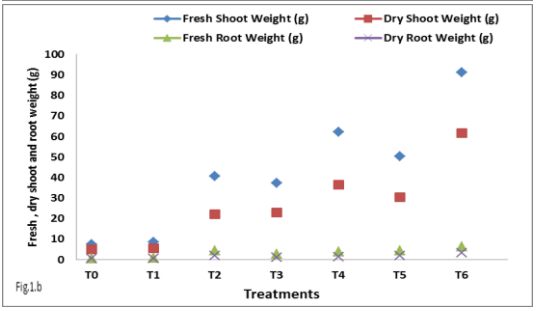


Fig 1 a and b: Effect of Carbendazim and solarized soil with *Pseudomonas fluorenscens*, spent mushroom compost on plant growth parameters of tomato plants at 150 days after treatment.

Table 2: Effect of Carbendazim and solarized soil with *Pseudomonas fluorenscens*, spent mushroom compost on disease intensity (%) and reduction (%) of Tomato Plants

Treatment		60 DAT		90 DAT		120 DAT		150 DAT	
		Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control
T0	*F. o* alone	23.7	0	36.2	0	49.9	0	65.3	0
T1	S.S + *F. o*	20.0	15.7	25.0	31.0	30.8	38.1	41.4	36.6
T2	S.S + s.m.c +*F. o*	10.0	57.8	21.2	41.3	25.0	49.8	26.0	60.2
T3	S.S + *P.f* +*F. o*	8.7	63.1	16.2	55.1	20.0	59.9	23.0	64.7
T4	S.S + s.m.c + *P.f* +*F. o*	7.5	68.4	15.0	58.6	18.7	62.4	21.2	67.4
T5	C +*F. o*	6.2	73.6	12.4	65.5	16.2	67.4	17.2	73.6
T6	Tomato plant alone	0.0	100	0.0	100	0.0	100	0.0	100
F- test		S		S		S		S	
S. Ed. (±)		6.044		5.179		4.789		4.998	
C. D.(P =0.05)		12.814		10.980		10.153		10.596	

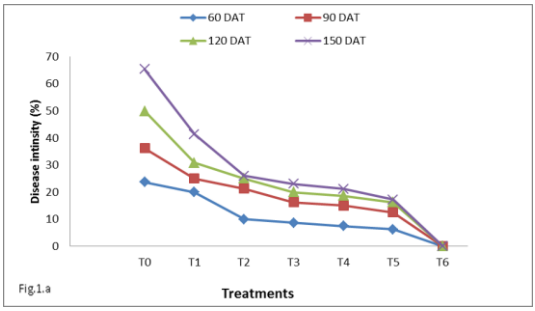
Solarized soil = S.S

Fusarium oxysporum = *F.o*

spent mushroom compost = s.m.c

Carbendazim = C

Pseudomonas fluorescens=*P. f*



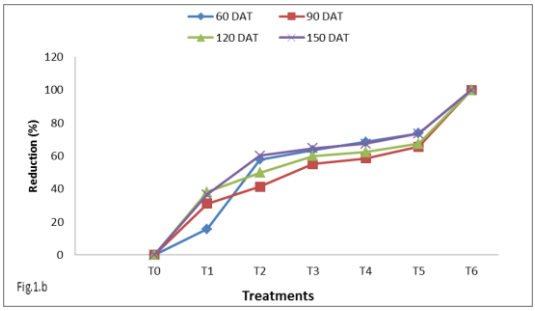


Fig 2 a and b: Effect of Carbendazim and solarized soil with *Pseudomonas fluorenscens*, spent mushroom compost on disease intensity (%) and reduction (%) of Tomato Plants

Discussions

Our finding are Similar with Dediel and Andréa,(2013) reported that microbiolization of seeds with (*Pseudomonas* sp.) reduced the tomato wilt in both assays (36.6 and 91.7% in the first and second assays respectively. Seleim *et al.* (2011) indicated that *Pseudomonas fluorescens* exhibited of tomato bacterial wilt disease (80%) followed by *Pseudomonas putida* . Saravanan *et al.* (2013) reported that inoculation with fulorescent *Pseudomonas* Pf5 induced a significant increase in shoot and root length of tomato plants. The infected plants with either *F. oxysporum* or *R. solani* drastically reduced the shoot length of the plant. However, in the presence of fluorescent *Pseudomonas* the adverse effect of the pathogens on growth of tomato plants was alleviated. Saravanan *et al.* (2013) reported an increase in

root and shoot weight of corn plants upon inoculation with a strain of *Pseudomonas putida* that also possess antagonistic activity against *Fusarium*. Abdullah *et al.* (2013) indicates that significantly increased the fresh root weight in treatment carbendazim (5.50g) as compared with control that recorded minimum fresh root weight (3.25g). Meenakshisundaram and Santhaguru, (2012) indicates that plants emerged from seeds inoculated with *Pseudomonas fluorescens* showed an increase in dry root weight compared to control plants. Seleim *et al.* (2011) *Pseudomonas fluorescens* recorded the highest increase percentage of yield per plant (348%) followed by *Pseudomonas putida* .

Plate 1: Effect of *F. oxysporum* on tomato plants with solarized soil (T1) and unsolarized soil (T0) at 150 day after germination

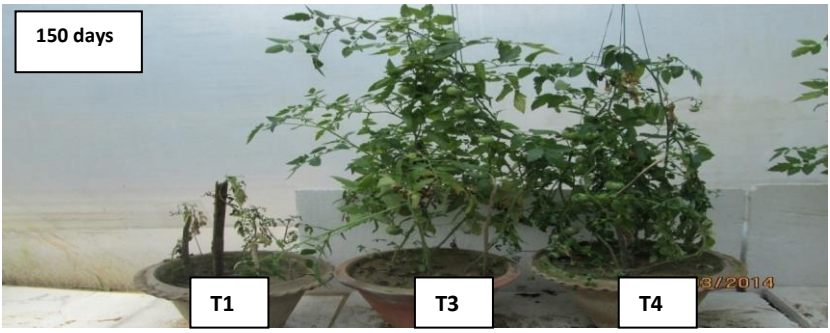


Plate 2: Effect of tretments (T1) *F. oxysporum*, (T3) S.S +*Pseudomonas fluorescens* and (T4) S.S +spent mushroom compost + *Pseudomonas fluorescens* on tomato plants at 150 day after germination

Plate 3: Effect of tretments (T0)Non S.S+ *F. oxysporum*, (T1) S.S+ *F. oxysporum* (T2) S.S +spent mushroom compost, (T3) S.S +*Pseudomonas fluorescens* , (T4) S.S +spent mushroom compost + *Pseudomonas fluorescens*, (T5) carbendazim + *F. oxysporum* and (T6) tomato plant alone on tomato plants at 120 day after germination

REFERENCES

Abdul Jaleel C, Gopi R, Panneerselvam R (2009). Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*. Plant Omics J. 2(1):30-40

Abdullah abid, Shfaat ahmed and Kamaluddeen, (2013).Efficacy of *Trichoderma* spp, Neem products and carbendazim against Fusarial wilt of tomato (*Lycopersicon esculentum* L) in pot conditions, International Journal of Agricultural Science and Research (IJASR) Vol. 3, Issue 5, Dec 2013, 73-80.

Armstrong G.H., and Armstrong J.K., (1981). *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. Pages 391- 399 in *Fusarium*: Disease, Biology and Taxonomy. P.E. Nelson,T.A.Toussoun and R.J.Cook, eds. Pennsylvania State University Press, University Park ,*Biotechnology* 23: 1503-1509.

De La Fuente L., Thomashow L., Weller D., Bajsa N., Quagliotto L., (2004). *Pseudomonas fluorescens* UP61 isolated from birdsfoot trefoil rhizosphere produces multiple antibiotics and exerts a broad spectrum of

biocontrol activity. *European Journal of Plant Pathology* 110: 671-681.

Dediel Júnior A. Rocha; Andréa B. Moura,(2013). Biological control of tomato wilt caused by *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp. *lycopersici* by rhizobacteria, Tropical plant pathology vol.38 no.5

Elad Y., (1995). Mycoparasitism. In: Kohmoto K., Singh U.S., Singh R.P. (eds). Pathogens and Host Specificity in Plant Diseases, II, Eucaryotes, pp. 285-307. Pergamon Press, Oxford, UK.

Errakhi R., Bouteau F., Lebrihi A., Barakate M., (2007). Evidences of biological control capacities of *Streptomyces* spp. Against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Beta vulgaris* L.). *World Journal of Microbiology and*

Esposito, R., and Fletcher, A. A. (1961). The relationships of pteridine biosynthesis to the action of copper 8-hydroxyquinolate on fungal spores. Arch. Biochem. Biophys. 93:369-376.

Karthikeyan M, Radhika K, Mathiyazhagan S, Bhaskaran R, Samiyappan R, Velazhahan R (2006). Induction of phenolics and defese-related enzymes in coconut (*Cocos nucifera* L.) roots treated with biological agents. Braz J Plant Physiol. 18(3):367-377

King E.O., Ward M.K., Ranney D.E., (1954). Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory and Clinical Medicine 44: 301-307.

Lukade G.M., (1992). Effect of organic soil amendments on root rot incidence of safflower, Madras Agric. J., 79: 179-181

Madi L., Katan T., Katan J., Henis Y. (1997). Biological control of *Sclerotium rolfsii* and *Verticillium dahliae* by *Talaromyces flavus* is mediated by different mechanisms. *Biological Control* 87: 1054-1060.

Martyn, R. D., and Hartz, T. K. (1986).Use of soil solarization to control Fusarium wilt of watermelon. Plant Disease 70:762-766.

Meenakshisundaram M and Santhaguru K,(2012). Biocontrol potential of fluorescent pseudomonas against wilt off tomato caused by *fusarium oxysporium,* International Journal of Science Innovations and Discoveries, Volume 2, Issue 1, January-February 2012 152-163 *Microbiology* 47: 153-158.

Saravanan T, Bhaskaran R, Muthusamy M (2004). *Pseudomonas flurescens* induced enzymological changes in banana roots against Fusarium wilt disease. Plant Pathology J. 3(2):72-80

Saravanan. S, Muthumanickam. P, Saravanan. T. S and Santhaguru. K, (2013).antagonistic potential of *fluorescent pseudomonas* and its impact on growth of tomato challenged with pathogens, African Crop Science Journal, Vol. 21, No. 1, pp. 29 – 36.

Seleim M.A.A., F.A. Saead, K.M.H. Abd-El-Moneem and K.A.M. Abo-ELyousr, (2011). Biological Control of Bacterial Wilt of Tomato by Plant Growth Promoting Rhizobacteria. Plant Pathology Journal, 10: 146-153.

Sheu Z.M., and Wang T.C.,(2006). First report of Race 2 of *Fusarium oxysporum* f.sp. *lycopersici*, the causal agent of fusarium wilt on tomato in Taiwan. AVRDC – the world vegetable center, tainan, Taiwan, R.O.C.Plant Dis., 90: 111
http://dx.doi.org/10.1094/PD-90-0111C

Singh A., Mehta S., Singh H.B., Nautiyal C.S., (2003). Biocontrol of collar rot disease of betelvine (*Piper betle* L.) caused by *Sclerotium rolfsii* by using rhizosphere-competent *Pseudomonas fluorescens* NBR I-N6 and *P. fluorescens* NBRI-N. *Current*

Singh R.D., and Amrit Sandhu, (1995). Factors affecting development of charcoal rot of cowpea. Abstract of paper

presented in global conference on advances in research on plant diseases and their management, Indian J. Mycol. Pl. Pathol., 25: 74

Tsahouridou P.C., Thanassoulopoulos C.C. (2002). Proliferation of *Trichoderma koningii* in the tomato rhizosphere and the suppression of damping-off by *Sclerotium rolfsii*. *Soil Biology and Biochemistry* 34: 767-776.