J. Biol. Control, 23(1): 31-36, 2009

Evaluation of biocontrol efficacy of *Trichoderma* isolates and methods of its application against wilt of chilli (*Capsicum annuum* L.) caused by *Fusarium solani* (Mart) Sacc.

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ABSTRACT: Seven isolates of *Trichoderma* spp. were evaluated as bio-agents against chilli wilt caused by *Fusarium solani* in the laboratory as well as pot culture experiments. Among the *Trichoderma isolates*, *T. viride* PDBCTV I0 recorded 100.00 per cent inhibition of linear growth of *F. solani* under dual culture on potato dextrose agar regardless of whether the *Trichoderma* spp. were seeded at all same time, one day prior, or two days prior inoculation (DPI) with the pathogen. *T. harzianum* PDBCTH I0 recorded 100.00 percent inhibition only in the 2 DPI treatments. The antagonists were applied in three different ways with or without carrier for optimization of delivery system. Seven days prior application (DPA) of bio agents favored maximum proliferation of propagules of antagonist in the soil followed by simultaneous application of antagonist and test pathogen and 1east number of propagu1es of antagonists were recorded in application through seed treatment. Under pot conditions, *T. harzianum* PDBCTH 10 and *T. viride* (*Indigenous*) recorded maximum number of propagules in soil. Further, it reduced *F. solani* propagules up to 100.00 per cent at 60 days after application (DAA), indicating number of test pathogen came down to essentially '0'. However, *T. viride* PDBCTV I0, *T. viride* PDBCTC 23, *T. viride* PDBCTV 24, Trieco (*T. viride)* and *T. harzianum* PDBCTH 8 also reduced *F. solani* population from 10.61 x 10^3 cfu g^{-1} of soil to less than 2 x 10^3 cfu g^{-1} in 7 DPA treatment at 60 DAA.

KEY WORDS: Application methods, bio agents, dual culture, formulation, *Fusarium solani*, *Trichoderma*, wilt.

INTRODUCTION

Although chemical control remains highly effective against plant diseases, developing countries with a low gross national product cannot utilize these products without placing an economic strain on national budget. There is also an intensified world wide concern about environmental pollution due to escalated use of hazardous pesticides. Furthermore, the development of resistance in pathogens against chemicals will force further restriction on the use of chemicals in sustainable control.

A multitude of microbes has been implicated as biocontrol agents of plant pathogens sometimes with excellent documentation. Antagonistic effect of rhizosphere on bottle gourd (Gaikwad, 1982) and a few of the studies on suppressive effect of some soils on muskmelon wilt (Alabouvette *et al.,* 1984; Sivan and Chet, 1986; Sharma, 1989) and watermelon wilt (Naik, 1990) demonstrate encouraging trends towards biological control. In recent years, the incidence of *Fusarium* wilt of chilli has increased in irrigated black cotton soil (Devika Rani *et al.* 2007). In the absence of resistant varieties, growers are left with few alternatives. Hence, studies were carried out to determine if effective bio-control agents for the wilt of chilli can be found and to evaluate different application methods so that bio-control techniques could be a useful component in the present day IDM program. Antagonists obtained from different sources were screened *in vitro* using dual culture with *F. Solani* and *in vivo* with pot culture experiments to test their efficacy against *F. solani.*

MATERIALS AND METHODS

Bio agents obtained indigenously and from the Project Directorate of Biological Control (PDBC), Hebbal, Bangalore was evaluated for their efficacy both *in vitro* using dual culture technique and *in vivo* using pot culture against *F. solani*. The mechanism of biocontrol was studied for selecting efficient bio agents.

Dual culture test

Seven isolates of *Trichoderma* (Table 1) were evaluated for their efficacy through dual culture technique. *Trichoderma* isolates and the test fungus were placed side by side on a single Petri dish containing solidified potato dextrose agar (PDA). Three treatments viz., simultaneous placement of pathogen and bio agents, one day prior to inoculation (DPI) of bio-agent and two DPI of bio-agents were tested. There were three replications for each isolate with one control that consisted of the pathogen or bio-agent.

Table 1. Source and identity of bio agents used against *F. solani*

S. N.	Bio-agents	Source
1.	*Trichoderma viride* PDBCHTV 24	PDBC, Bangalore
2.	*T. viride* PDBCHTV 23	PDBC, Bangalore
3.	*T. harzianum* PDBCTH 8	PDBC, Bangalore
4.	*T. harzianum* PDBCTH 10	PDBC, Bangalore
5.	*T. viride* PDBCTV 10	PDBC, Bangalore
6.	*T. viride* (Indigenous)	Biocontrol unit, Raichur
7.	Trieco (*T. viride*)	Ecosense labs (I) Pvt. Ltd., Mumbai

They were incubated for 6-7 days. The diameter of the colony of both bio-agent and the pathogen was measured in two directions and the average was calculated. Per cent inhibition of growth of the test pathogen was calculated by using the formula (Naik and Sen, 1994).

Multiplication of *F. solani* for soil application

The fungus was grown on sterile sorghum grain medium at 28 ± 1°C for three weeks. The medium was prepared by soaking the surface sterilized sorghum grains for over night with 2 per cent sucrose solution and water drained off. Grains (200g) were autoclaved half an hour for three consecutive days. The sterilized sorghum grains were seeded with an agar plug colonized by *F. solani* and incubated for three weeks. The inoculum was added to the sterilized soil 10% [W/W basis] to make soil sick. The inoculum from such mixture was enumerated and expressed as colony forming units per gram (CFUs g^{-1}) of soil.

Application of *Trichoderma* spp. to *Fusarium* infested soil

Simultaneous application (SA)

The mixture of *Trichoderma* and *Fusarium* were incorporated in plastic pots of 10cm diameter, containing sterilized soil simultaneously. The same procedure was followed for different isolates and incubated for three days. Soil moisture of 50 per cent water holding capacity was maintained in pots by watering regularly.

Seven day prior application (DPA) of bio-agent

In another study, *Trichoderma* isolates grown on FYM were applied to sterilized soil seven DPA with *Fusarium* for each isolate of *Trichoderma*. *F. solani* was then added to the same pots mixed thoroughly and incubated for three days. Soil moisture of 50 per cent water holding capacity was maintained by watering regularly.

iii) Application through seed treatment (ST)

Trichoderma isolates grown on PDA were flooded with sterile water to prepare spore suspensions. Spore concentrations were adjusted to 5-6 x 10^8 conidia ml^{-1}. Chilli seeds were surface sterilized with $HgCl_2$ (0.1%) soaked in bio-agent spore suspension for two hours then air dried. Twenty five seeds were sown in each pot (10cm diameter) containing the infested soil described above. The pots sown with seeds soaked in sterile water served as control. The number of colony forming units (cfu) of both *Trichoderma* and *F. solani* per gm of soil sample were counted on respective specific media at 15 days interval up to 60 days.

Enumeration of antagonist and pathogen

Population densities of *Trichoderma* spp. and *F. solani* from the sampled soils were assessed on *Trichoderma* selective medium with captan (TSMC) (Elad *et al*., 1981) and *Fusarium* selective medium (FSM) (Nash and Snyder, 1962) respectively at 15 days interval up to 60 days after application (DAA) and per cent reduction in colony forming units of *F. solani* due to introduction of *Trichoderma* spp. was recorded. It was possible to monitor the respective population of pathogen and bio-agent only up to 60 days since it was a pot culture experiment.

RESULTS AND DISCUSSION

Dual culture test

All the *Trichoderma* isolates that were tested showed considerable growth inhibition of *F. solani.* Among the *Trichoderma* isolates, *T. viride* PDBCTV I0 caused 100.00 per cent inhibition of radial growth of *F. solani* in dual culture at all the inoculation periods at the end of 6th day, *T. harzianum* PDBCTH I0 recorded 100.00 percent inhibition only in 2 DPI at the end of 6th day of observation. Maximum of 100.00 per cent inhibition was observed in *T. viride* PDBCTV 10 followed by *T. harzianum* PDBCTH 10 (82.90 %), *T. viride* (Indigenous) (64.45 %) and *T. viride* PDBCHTV 24 (62.65 %) respectively. Lowest reduction (< 60%) was observed in three isolates [*T. viride* PDBCTV

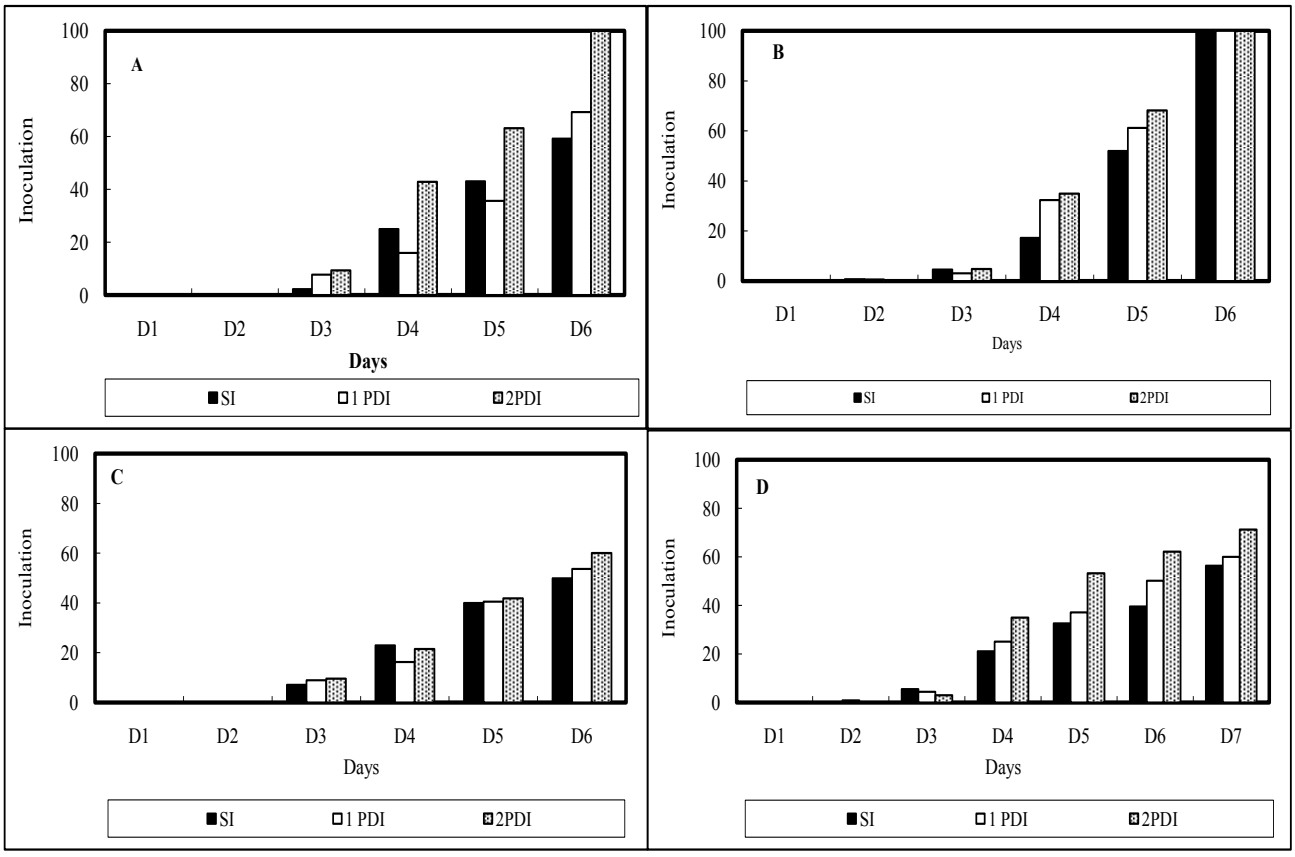

Fig 1. Effect of different inoculation periods of bioagents A (*Trichoderma* PDBCTH 10), B (*T. viride* PDBCTV 10), C [Trieco (*T. viride*) and D (*T. viride* PDBCTV 24) on inhibition of *F. solani*

23, and Trieco (*T. viride*)]. (Fig 1 and 2). Maximum linear growth inhibition of *F. solani* was recorded at 2 DPI by *Trichoderma* isolates tested.

The biocontrol mechanism might be due to competition, over growth and inhibition of growth of *F. solani.* There might be some antibiotic present in these antagonists responsible for exercising such adverse effect on germination of conidia of *F. solani.* Such mechanisms have been described by previous workers (Cook and Baker 1983; Mohamad Charif and Benhamou, 1990; Naik *et al.,* 2000; Howell, 2003 and Devika Rani, 2006). Antibiosis, competition and mycoparasitism have been noted to be effective mechanisms in *Trichoderma* plant pathogen interactions (Sen, 2000; Harman *et al*., 2004; Druzhinina *et al.,* 2006 and Singh, 2006). The fast growth observed in the present study may explain the competitive ability of such species against pathogenic *F. solani.* In addition, they are observed to be early colonizers of substrates and known to reduce the activity of other fungi simply by substrate occupation and depletion. Several possible microbial interactions have been observed that stimulate, inhibit, mutual intermingling, over growing of micro flora iso1ate and over growing of test pathogen or inhibition at a distance have been documented (Sharma, 1989; Naik and Sen, 1994; Harman *et al*., 2004).

Soil inoculation test

Effective delivery of antagonists has been a major challenge in the implementation of biocontrol as a management strategy. Several techniques have been tested for delivery of antagonists such as direct application through liquid broths (Kerr, 1980 and Marois *et al.,* 1982), use of organic matter like saw dust (Duda and Sierota, 1987 and Naik, 1990) use of vermiculture or pyraz clays (Fravel *et al.,* 1983), seed soaking (Siven and Chet, 1986) and root dippings (Yuen *et al.,* 1983). Seven DPA of bioagent favoured maximum proliferation of number of propagules of antagonist in the soil column followed by soil application of antagonist and test pathogen and 1east number of propagu1es of antagonists were recorded in application through seed treatment method.

Direct application of antagonists through FYM as a carrier either seven day prior or simultaneously resulted in build up of maximum number of antagonistic population. Hence, both the 7 DPA and simultaneously applied (SA) treatments recorded maximum number of propagules of bioagent. farm yard manure might provide extra nutrition for the growth of antagonist. Multiplication of antagonist has been reported to be efficient on organics (Siven and Chet, 1986; Duda and Sierota, 1987; Naik 1990, Mandhare

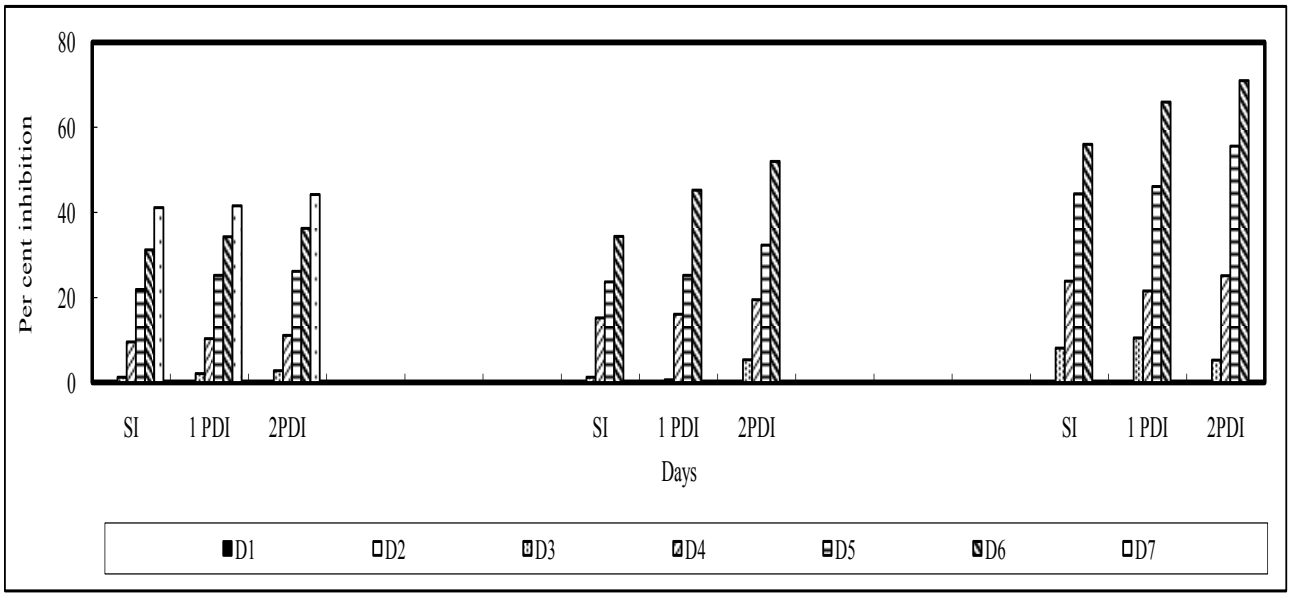

Fig 2. Effect of different inoculation periods of bioagents E (*T. viride* PDBCTV 23), F (*T. harzianum* PDBCTH 8), G [*T. viride* (indigenous)] on inhibition of *F. solani* growth

and Suryawanshi, 2005). While the number of propagules of antagonists increased, there was a proportionate reduction in propagules of test pathogen.

Among the antagonists tested under pot conditions, *T. harzianum* PDBCTH 10 and *T. viride* (indigenous) recorded maximum number of propagules in soil column. Further, it reduced the test pathogen (*F. solani*) up to 100.00 per cent at 60 DAA. That means at 60 DAA the number of *F. solani* came down to essentially '0', which was a remarkable reduction in population of pathogen. However, *T. viride* PDBCTV I0, *T. viride* PDBCTV 23, *T. viride* PDBCTV 24, Trieco (*T. viride)* and *T. harzianum* PDBCTH 8 also reduced soil densities of *F. solani* population from 10.61 x 10^3 cfu g^{-1} of soil to less than 2 x 10^3 cfu g^{-1} of soil in 7 DPA treatment at 60 DAA, indicating reduction up to 80 per cent of pathogenic population (Fig 3 and 4). We found that fast multiplication of antagonists was maximum in FYM and maximum reduction in the propagules of *F. solani* was highest in 7 DPA of bio-agent followed by soil application. The lowest reduction in propagules was recorded when seeds were inoculated with the antagonists. Of all the bio-agents tested, soil application of antagonists applied with FYM was found to be superior to that seed application of antagonists. Antagonists proliferated up to 60 days was enough for bringing down the test pathogen population to significantly low level.

Soil application through FYM as a carrier provides the maximum reduction of test pathogen. This may be due to the maximum multiplication of bioagent on carrier that favoured its competitive mechanism of biocontrol. Seed inoculation with the antagonist also recorded good inhibition of test pathogen when it was used alone (Mandhare and Suryawanshi, 2005) by seed soaking and dry seed coating of *T. harzianum* against *Rhizoctonia solani* (Hwang and Benson, 2002), *F. oxysporum* f. sp. *vasinfectum* in cotton, *F. oxysporum* f. sp. *melonis* in melon (Sivan and Chet, 1986). Effect of *Trichoderma* against *Fusarium* and *Sclerotium rolfsii* (Monaco *et al.* 1991), as well as *T. viride* and *Gliocladium virens* against *F. oxysporum* f. sp. *ciceri* (Jha and Singh, 2000) have been documented successfully.

Application of antagonists through FYM and seed inoculation resulted in reduction in the propagules of test pathogen to a maximum extent. *T. harzianum* PDBCTH l0 when applied through FYM seven days in advance reduced the *F. solani* propagules from 8.10 X 10^3 cfu g^{-1} of soil just at 15 DAA and a conspicuous reduction of 10.63 X 10^3 cfu g^{-1} of soil to zero level was witnessed at 60 DAA. *T. viride* (Indigenous) also recorded almost similar result.

Considering the increase in bioagent population and the reduction in population of *F. solani* densities, FYM as a carrier for all *Trichoderma* isolates was adjudged as the best method for formulation and delivery of antagonists to infested soil to suppress the pathogen population.

Hence, biological control through the use of *Trichoderma* antagonists has been found to be potential in destruction of *F. solani* inoculum causing wilt of chilli. In the absence of resistant cultivars, use of such potential bio agents would go a long way in mitigating Fusarium wilt thus favoring sustained chilli production in infested fields. The biocontrol technology is eco-friendly and free from environmental hazards since it avoids soil application of chemical to manage such soil borne disease.

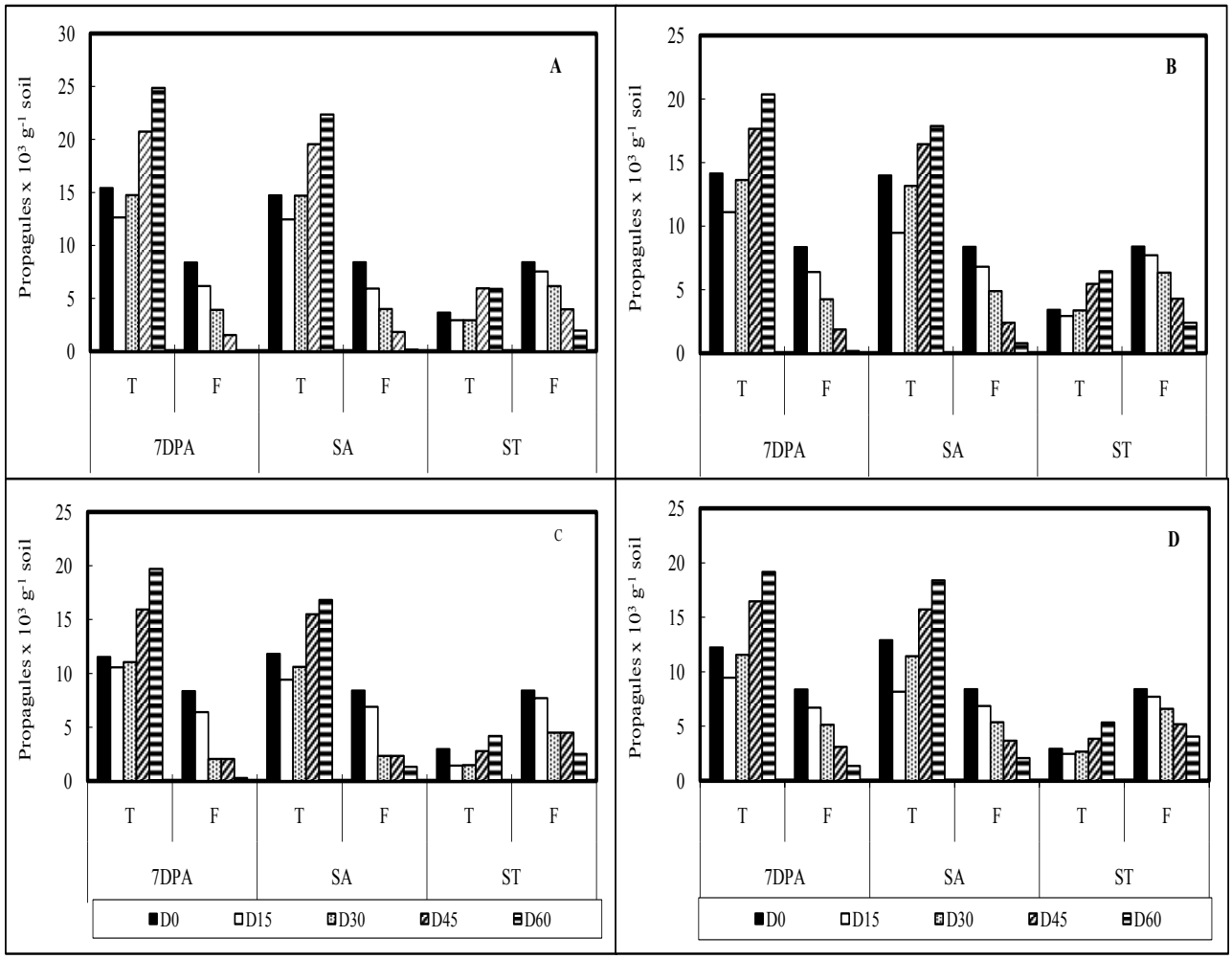

Fig 3. Effect of different application methods of bioagents A (*Trichoderma* PDBCTH 10), B (*T. viride* PDBCTV 10), C [Trieco (*T. viride*)] and D (*T. viride* PDBCTV 24) on soil propagules of *F. solani* (7DPA: 7 days prior application of bioagents; SA: Simultaneous application of bioagents and *Fusarium*; ST: Seed treatment with bioagent)

REFERENCES

Alabouvette, C., Couteaudier, Y. and Louvet, J. 1984. Dynamique des populations de *Fusarium* spp. Et de *Fusarium oxysporum* f. Sp. *Melonis* doans un soil resistant et dans in soil sensibleaux fusarioses vascularies. *Agronomie,* **4**: 729-733.

Bineeta Sen, 2000. Biological control: A success story. *Indian Phytopathology*, **53**: 243-249.

Cook, R. J. and Baker, K. F. 1983. *The nature and practice of biological control of plant pathogens*. American Phytopathological Society, St. Paul, Minnesota, 539p.

Devika Rani, G. S. 2006. *Towards promoting disease resistance and biological control of Fusarium wilt of chilli.* M.Sc (Agri) Thesis. University of Agricultural Sciences, Dharwad.

Devika Rani, G. S., Naik, M. K., Raju, K. and Prasad, P. S. 2007. Prevalence of wilt of chilli and assessment of population dynamics of *Fusarium* in predominant chilli (*Capsicum annuum* L.) growing regions of Karnataka. *Journal of Soil Biology and Ecology*, **27**: 50-61.

Druzhinina, I. S., Schmoll, M., Seiboth, B. and Kubicek, C. P. 2006. Global carbon utilization profiles of wild-type strains, mutants and transformants of *Hypocrea jecorina. Applied and Environmental Microbiology*, **72**: 2126-2133.

Duda, B. and Sierota, Z. H. 1987. Survival of scots pine seedlings and chemical of damping off in plastic green house. *European Journal of Forest Pathology,* **17**: 110-117.

Elad, Y., Chet, I. and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* sp. from soil. *Phytoparasitica,* **9**: 59-67.

Fravel, D. R., Papavizas, G. C. and Marois, J. J. 1983. Survival of ascospores and conidia of *Talaromyces flavus* in field soil and pyrax. *Phytopathology,* **73**: 821.

Gaikwad, S. J. 1982. Management of cucurbit wilt caused by *Fusarium oxysporum* Scwecht., Ph.D. Thesis, IARI, New Delhi, 112p.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M., 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Review of Microbiology,* **2**: 43-56.

Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant disease: The history and evolution of current concepts. *Plant Disease,* **87**: 4-10.

Hwang, J. and Benson, D. M. 200l. Resistance of poinsettia cultivars against *Rhizoctonia* stem rot induced by binucleate *Rhizoctonia*. *Phytopathology.* **91**: 541.

Jha, D. K. and Singh, D. K. 2000. Biological control of chickpea wilt. In: *Proceedings of International Conference on Integrated Plant Disease Management for Sustainable Agriculture.* Vol. I: 321. Indian Phytopathological Society, IARI, New Delhi, 665p.

Kerr, A. 1980. Biological control of crown-gall through production of agrocin 84. *Plant Disease,* **64**: 25-30.

Mandhare, V. M. and Suryawanshi, A.V. 2005. Standardization of storage conditions to increase the shelf life of *Trichoderma* formulations. *Agricultural Science Digest,* **25**: 71-72.

Marois, J. J., Johnston, S. A., Dunn, M. T. and Papvizas, G. C. 1982. Biological control of Verticillium wilt of egg plant in field. *Plant Disease,* **66**: 1166.

Mohamed Cherif and Benhamou, N. 1990. Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis lycopersici. Phytopathology,* **80**: 1406-1414.

Monaco, C., Perello, A., Allippi, H. E. and Pasquare, A. O. 1991. *Trichoderma* spp. and *Sclerotium rolfsii* by seed treatment. *Advances in Horticultural Sciences,* **5**: 92-95.

Naik, M. K. 1990. Wilt of watermelon caused by *Fusarium oxysporum* Schlecht. Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, pp. 184.

Naik, M. K and Sen, B. 1994. Effectiveness of biocontrol agents against a spectrum of *Fusarium* isolates causing wilt of watermelon. *Indian Journal of Plant Protection,* **21**: 19-22.

Naik, M. K. and Sen, B., 1995. Biocontrol of plant disease caused by *Fusarium* spp. p. 32. In: Mukerjee, K.G. (Ed), *Recent Developments in Biocontrol of Plant Diseases*. Aditya Publishing House, New Delhi, India.

Naik, M. K., Singh, S. J. and Sinha, P. 2000. Mechanism of biocontrol of wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici.* Proceedings, Golden Jubilee International Conference on Integrated Plant Disease Management for Sustainable Agriculture. Vol. 1: 401-402. Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi, 665p.

Nash, S. M. and Snyder, W. C. 1962. Quantitative estimation by plate counts of propagules of the bean root rot *Fusarium* in field soil. *Phytopathology,* **51**: 567-572.

Sharma, J. 1989. *Spectrum and application of the antagonist in the biological control of Fusarium wilt of cucurbits.* Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, 123p.

Singh H. B. 2006. *Trichoderma*: A boon for biopesticides industry. *Journal of Mycology and Plant Pathology,* **36**: 373-384.

Sivan, A. and Chet, I. 1986. Biological control of *Fusarium* species in cotton, wheat and muskmelon by *Trichoderma harzianum. Phytopathology.* **116**: 39-47.

Yuen, G. Y., Mc Cain, A. H. and Schroth, M. N. 1983. The relation of soil type to suppression of *Fusarium* wilt of carnations. *Acta Horticulturae,* **141**: 95-102.

(Received: 12-01-2008; Revised: 25-09-2008; Accecpted: 27-11-2008)