Plant Protection Journal
Vol. 1, No. 1, 2009 : 108- 113

Efficacy of some fungal and bacterial bioagents against *Fusarium oxysporum* f.sp. *ciceri* on chickpea

Hesamedin RAMEZANI*
Department of Agriculture, Payame Noor University (P.N.U.), Shiraz, Iran

Abstract

The efficacy of four fungal bioagents viz., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma viride*, *Gliocladium virens* and two bacterial bioagents namely *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against the chickpea vascular wilt pathogen, *Fusarium oxysporum* f.sp. *ciceri in vitro* condition using Dual Culture Technique. Among the fungal bioagents, *T. harzianum* produced the maximum inhibition zone of 17 mm compared to the minimum of 7 mm by *T. hamatum*. There was no significant difference between the inhibition zones produced by *P. fluorescens* and *B. subtilis*. Soil application of talc- based formulation of *T. harzianum*, *P. fluorescens* and *G. virens* effectively controlled the vascular wilt of chickpea under field condition.
Key words: Biological control, Chickpea, Vascular wilt, Bioagents.

Introduction

Among various pulses crops, chickpea (*Cicer arietinum* L.) is considered as one of the oldest one cultivated both in Asia and Europe. Like many other crops, pulses, especially chickpea have also been reported to suffer sever yield losses due to various insect pests and diseases. Among different diseases, fungi especially, the wilt caused by species of *Fusarium* remains to be a challenging task in terms of management since it is soil- borne in nature (Agrios, 2000; Butler, 1918; Singh *et al.*, 1986).

Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods. All these methods are effective only when employed well in advance as precautionary measure (Kata, 2000; Sharma, 1996). Once a disease has appeared, these methods become impractical / ineffective. In that situation, chemical control offers a good choice to grower to control the disease. Chemical pesticides have been in use since long and they provide quick, effective and economic management of plant diseases. However, in recent past, it has been

* Corresponding author, Email: hesami18@yahoo.com
Received: 4 October 2008, Accepted: 30 December 2008

realised that use of chemicals in agriculture is not as beneficial as it was visualised. Chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment (Hayes & Laws, 1991). Increasing awareness of humankind toward the ecosystem and environment has made a marked shift from synthetic materials to bio- products. They are biodegradable, eco-friendly, relatively broad spectrum, no possibility of anthropogenic diseases and resistance problem in target organism is eliminated. Effectiveness of various fungal (Hadar *et al.*, 1979; Kim & Roh, 1987; Lifshitz *et al.*, 1986; Papavizas & Lewis, 1989; Tu, 1991) and bacterial (Raguchander *et al.*, 1998; Vidhyasekaran *et al.*, 1997) bioagents against plant pathogens have been reported. Hence, a study was initiated to investigate the efficacy of certain fungal and bacterial biocontrol agents against *F. oxysporum* f.sp. *ciceri* under laboratory and field conditions.

Material and Methods

The pathogen and bioagents used in the present study were obtained from the Division of Mycology and Plant pathology, IARI, New Delhi, India. One– week old culture of pathogen and bioagents maintained on PDA slants at 25 ± 2^{o}C were used for the present study. Biological activity of these bioagents was determined by Dual Culture Technique (Dennis & Webster, 1971). In this method, both the pathogen and bioagents from periphery of 5 days old culture were inoculated on PDA in a single Petri plate at a distance of 1 cm apart. In case of bacterial bioagents instead of placing a mycelial disc as a fungal bioagents, they were streaked using a flam sterilized inoculation needle. Each treatment was replicated four times and incubated at 25 ± 2^{o}C. Growth of both bioagents, pathogen and zone of inhibition as recorded after 7 days of inoculation.

Preparation of commercial formulation of bioagents

The commercial formulation of *Trichoderma* species and *Gliocladium virens* was prepared as per the procedure described by Jeyarajan & Ramaksirhnan (1991). In this regard, the culture of pathogen and bioagents were prepared on PDA in 250 ml conical flasks. After inoculation, the flasks were incubated at 25 ± 2 ^{o}C. The mycelial mat of pathogen and bioagents were harvested after 7 days of inoculation and blended in a known volume of distilled water in an electric blender and finally using talc- powder as carrier. Talc-based formulation of *Pseudomonas fluorescens* and *Bacillus subtilis* were prepared by method of Vidhyasekaran & Muthamilan (1995), in which the same process has been employed through growing the bacteria in nutrient broth and using talc as carrier.

Field efficacy of fungal and bacterial bioagents

A field trial was conducted in the sick plot at Soltanabad farm, Shiraz during February-May, 2007. Chickpea cultivar *Kaboli* was used. Talc- based formulation of the bioagent was incorporated in to the soil @ 2.5 Kg / ha at the time of

transplanting the seedling at 30 cm apart in a bed size of 5 m × 2 m. An untreated control was also maintained with three replications for each treatment. Observation on inhibition zone and wilt incidence were recorded and analyzed statistically using One- way ANOVA followed by Duncan's Multiple range test (Duncan, 1955).

Results and Discussion

The data presented in Table 1 indicated that all the six bioagents inhibited the growth of *F. oxysporum* f.sp. *ciceri* after 7 days of inoculation. *T. harzianum* exhibited the maximum biocontrol activity causing an inhibition zone of 17 mm followed by *G. virens*, *T. viride* and *T. hamatum* causing 14, 10 and 7 mm inhibition zones, respectively. *In vitro* condition, among the three species of *Trichoderma*, the maximum effect was noticed by *T. harzianum*, while *T. hamatum* showed the minimum biocontrol activity against chickpea vascular wilt pathogen. However, differences among these species in terms of inhibitory effect were statistically highly significant (Table 1). The inhibitory effect of *T. hamatum* recorded half of an inhibition zone causing by *G. virens*. The effect of two bacterial bioagents *P. fluorescens* recorded an inhibition zone of 13 mm as against 12 mm by *B. subtilis*, which were statistically insignificant. The inhibitory effect of these fungi against *F. oxysporum* f.sp. *ciceri* was probably due to competition and/or antibiosis. The biocontrol activity of these bioagents observed in the present study is similar to the finding of Hadar *et al.* (1979), Mathew & Gupta (1998) and Rajappan & Ramaraj (1999), who reported effective inhibition of *Rhizoctonia solani* and *Fusarium moniliforme*. On the other hand, Kim & Roh (1987) also observed the same biocontrol activity of *T. harzianum*, *T. viride* and *G. virens* against *R. solani*. Benhamou & Chet (1993) were also reported the same efficacy of *T. harzianum* against *R. solani*.

Table 1- *In vitro* efficacy of bioagents against *Fusarium oxysporum* f.sp. *ciceri*

Bioagents	Growth (mm)		Inhibition zone (mm) †
	Bioagents	Pathogen	
Trichoderma hamatum	65	18	7 [e]
Trichoderma harzianum	58	15	17 [a]
Trichoderma viride	63	17	10 [d]
Gliocladium virens	66	10	14 [b]
Pseudomonas fluorescens	12	63	13 [c]
Bacillus subtilis	34	44	12 [c]
Pathogen alone	-	90	-

† Different alphabets in column represent insignificant difference at $p < 0.05$ employing DMRT.

Table 2- Field evaluation of bioagents against *Fusarium oxysporum* f.sp. *ciceri* on chickpea

Bioagents	Percent Wilt Incidence †
Trichoderma hamatum	15 [b]
Trichoderma harzianum	5 [d]
Trichoderma viride	14 [b]
Gliocladium virens	7 [c]
Pseudomonas fluorescens	6 [c]
Bacillus subtilis	18 [b]
Pathogen alone	33 [a]

†Different alphabets in column represent insignificant difference at $p < 0.05$ employing DMRT

Under filed condition, *T. harzianum* recorded the lowest wilt incidence of 5 percent, while *B. subtilis* showed the greatest wilt incidence of 18 percent (Table 2). Among the three species of *Trichoderma* under field condition, *T. harzianum* showed the minimum wilt incidence compared to the *T. hamatum* and *T. viride*. The effect of two bacterial bioagents *P. fluorescens* and *B. subtilis* recorded wilt incidence of 6 and 18 percent, respectively. However, differences between these two bioagents were noticed statistically highly significant. On the other hand, the effect of biocontrol activity of *T. hamatum*, *T. viride* and *B. subtilis* were statistically insignificant in terms of wilt incidence (Table 2). The effect of biocontrol activity of these bioagents against chickpea vascular wilt pathogen in the present study is similar to the finding of Chet & Baker (1981) and Chand *et al.* (1991). Coley-Smith *et al.* (1991) also reported that bottom-rot disease of lettuce was suppressed by *T. harzianum* and *T. viride in vivo* condition. Vidhyasekaran *et al.* (1997) obtained effective control of pigeon pea wilt caused by *F. udum* using talc- based formulation of *P. fluorescens*. Raguchander *et al.* (1998) reported that seed pelting with *B. subtilis* effectively controlled soybean root- rot caused by *Macrophomina phaseolina* and increased the grain yield.

The present study clearly indicates the efficacy of *T. harzianum*, *G. virens* and *P. fluorescens* against *F. oxysporum* f.sp. *ciceri* can be exploited under field condition to manage the disease.

Reference

Agrios, G.N. 2000. Significance of plant disease, pp. 25-37, In: Agrios, G.N. (Ed.), *Plant Pathology*. Academic Press, London.

Benhamou, N. & Chet, I. 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*. Ultra structure and gold cytochemistry of mycoparasitic process. *Phytopathology*, 83(10): 1062-1071.

Butler, E.J. 1918. *Fungi and Diseases in Plants*. Thacker Spink Co., Culcutta, India.

Chand, H., Chhabra, M.L. & Jalali, B.L. 1991. Promising biocontrol agents for the control of chickpea wilt. *Indian Phytopathology*, 46: 36-39.

Chet, I. & Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive of *Rhizoctonia solani* under field condition. *Phytopathology*, 71(3): 286-290.

Coley- Smith, J.R., Ridout, C.J., Mitchell, C.M. & Lynch, J.M. 1991. Control of bottom-rot disease of lettuce (*Rhizoctonia solani*) *in vivo* condition by using preparation of *Trichoderma viride* and *T. harzianum* or tolclofosmethyl. *Plant Pathology*, 40: 359-366.

Dennis, C. & Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma* III. Hyphal interaction. *Transactions of the British Mycological Society*, 57: 363-369.

Duncan, D.B., 1955. Multiple range and multiple F-tests. *Biometrics*, (2-4): 1- 42.

Jeyarajan, R. & Ramaksirhnan, R. 1991. Efficacy of *Trichoderma* formulation against root rot disease of grain legumes. *Petria*, 1: 137-138.

Hadar, Y., Chet, I. & Heins, Y. 1979. Biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum*. *Phytopathology*, 69: 64-68.

Hayes, W.J. & Laws, E.R. 1991. *Handbook of Pesticide Toxicology*. Vol. 1. Academic Press Inc., New Delhi.

Kata, J. 2000. Physical and cultural methods for the management of soil borne pathogens. *Crop Protection*, 19: 725-731.

Kim, H.K. & Roh, M.J. 1987. Isolation, identification and evaluation of biocontrol potential of rhizosphere antagonists to *Rhizoctonia solani*. *Korean Journal of Plant Protection*, 26: 26-87.

Lifshitz, R., Windham, M.I. & Baker, R. 1986. Mechanism of biological control of pre-emergence damping off of pea by seed treatment with *Trichoderma* species. *Phytopathology*, 76(7): 720-725.

Mathew, K.A. & Gupta, S.K. 1998. Biological control of root rot of french bean caused by *Rhizoctonia solani*. *Journal of Mycological Plant Pathology*, 28: 202-205.

Papavizas, G.C. & Lewis, J.A., 1989. Effect of *Gliocladium* and *Trichoderma* on damping off and blight of snapbean caused by *Sclerotium rolfsii* in green house. *Plant Pathology*, 38: 277-286.

Raguchander, T., Rajappan, K. & Samiappan, R., 1998. Influence of biocontrol agents and organic amendment on soybean root rot. *International Journal of Tropical Agriculture*, 35: 23-29.

Rajappan, K. & Ramaraj, B. 1999. Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing wilt of cauliflower. *Annals of Plant Protection Society*, 7(2): 205-207.

Sharma, P.D.1996. *Plant Pathology*. Rastogi Publ., Meerut, India.

Singh, G., Kapoor, S., Gill, A.S. & Singh, K. 1986. Chickpea varieties resistant to *Fusarium* wilt and food-rot. *Indian Journal of Agricultural Sciences*, 56(5): 344-346.

Tu, J.C. 1991. Comparison of the efficacy of *Gliocladium virens* and *Bacillus subtilis* in the control of seed rot and root rot navy beans. *Mededelingen Van de Faculteit Landbourn Weteschappen Rijks Universiteit Gent,* 56: 229-234.

Vidhyasekaran, P. & Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Disease*, 79: 782-786.

Vidhyasekaran, P., Sethuraman, K., Rajappan, K. & Vasumathi, K. 1997. Powder formulation of *Pseudomonas fluorescens* to control pigeon pea wilt. *Biological Control*, 8: 166-171.

تاثیر چند قارچ و باکتری کنترل کننده علیه قارچ *Fusarium oxysporum* f.sp. *ciceri* در نخود

حسام الدین رمضانی

گروه کشاورزی، دانشگاه پیام نور شیراز

(پست الکترونیکی: hesam18@yahoo.com)

چکیده

اثر چهار قارچ کنترل کننده *Trichoderma hamatum, Trichoderma harzianum, Trichoderma viride, Gliocladium virens* و دو باکتری کنترل کننده *Pseudomonas fluorescens* و *Bacillus subtilis* روی قارچ عامل بیماری پژمردگی آوندی نخود ایرانی (*Fusarium oxysporum* f.sp. *ciceri*) در شرایط آزمایشگاهی مورد بررسی قرار گرفت. در بین قارچهای کنترل کننده، *T. harzianum* با ۱۷ میلیمتر حداکثر کاهش در مقایسه با ۷ میلیمتر توسط *T. hamatum* کمترین کاهش در رشد عامل بیماری گزارش گردید. از لحاظ آماری میزان کاهندگی / کنترل کنندگی رشد قارچ عامل بیماری پژمردگی نخود در بین دو باکتری مورد استفاده در آزمایش تفاوت معناداری مشاهده نگردید. در شرایط مزرعه قارچهای *T. harzianum* و *G. virens* و باکتری *P. fluorescens* که به فرم ماده افزودنی پودر تالک استفاده گردیدند کاهش قابل توجه ای از لحاظ بیماری پژمردگی نخود در مقایسه با تیمار شاهد ایجاد کردند.

واژه‌های کلیدی: کنترل بیولوژیکی، نخود، بیماری پژمردگی، اثر بازدارندگی