

EFFICACY OF BIOAGENTS AGAINST CHICKPEA WILT PATHOGEN

S.Ranjitha Rani and S.S.Mane

Department of Plant Pathology, Post graduate Institute, Dr. PDKV, Akola 444001

Email: s.ranjithareddy@gmail.com

ABSTRACT: Chickpea (*Cicer arietinum* L), wilt caused by *Fusarium oxysporum f.sp. ciceri* was first reported from India in 1918. In the recent years, the biological control has received a worldwide attention and is being integrated effectively with other pesticides. The efficacy of two species of fungal bioagents such as *Trichoderma viride*, *Trichoderma harzianum* and two sps of bacterial bioagents such as *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Fusarium oxysporum f.sp. ciceri* *in vitro* condition using Dual Culture Technique. The highest per cent growth of inhibition of observed *Trichoderma harzianum* (76.66%) followed by *Bacillus subtilis* (63.14%). The lowest growth inhibition was observed in *Pseudomonas fluorescens* (53.52%).

Key words: Chick pea, *Fusarium oxysporum f.sp. ciceri*, Bioagents, *In vitro*.

INTRODUCTION

Chickpea is the world's third most important legume. India is the principle chickpea producing country. The wilt of chickpea incited by *Fusarium oxysporum f.sp. ciceris* is one of the serious diseases (Gupta *et al.*, 1986). This pathogen is soil borne (Singh *et al.*, 2009) and seed borne (Haware *et al.*, 1978) cause profound losses (20 to 100%) depending upon phase of illness and wilting (Haware and Nene, 1980). Chemical management of its infection by systemic fungicides is extravagant, but also causes ecological problem. Thus, the present investigation was planned to isolate, identify pathogen from infected plants of chick pea and evaluate *in vitro* the efficacy of different bioagents against *Fusarium oxysporum f.sp. ciceris*.

MATERIAL AND METHODS

Chickpea wilted plants were collected from pulses research unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, (P.D.K.V.), Akola. Common laboratory media i.e. potato dextrose agar was used for the isolation of the pathogen associated with the wilt of chickpea. The isolates of *Trichoderma* species (*T. viride*, *T. harzianum*) *Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from Department of Plant Pathology, Dr. P.D.K.V., Akola.

Isolation of pathogen from chickpea wilted plants:

Chickpea plant showing typical wilt symptoms were collected from the Field of Department of Plant Pathology, Dr. PDKV, Akola (M.S). The repeated isolations were made to isolate pathogen from wilted plants. The roots and stem of infected plants were washed in running tap water to remove soil before isolation to avoid contamination. The roots were cut into small bits of the size 2.5 mm, with sterilized blade. These bits were then surface sterilized with 0.1 per cent mercuric chloride for two minutes and washed with three changes of sterilized water to remove traces of mercuric chloride. Each bit was blot dried and four bits each placed on the solidified potato dextrose agar (PDA) plates. These plates were then incubated at 27±1°C for seven days. The fungal growth was transferred to the plates of PDA.

Efficacy of bioagents against *Fusarium oxysporum f.sp. ciceri* by dual culture technique.

The lawn culture of fungal bioagent *T. viride* and *T. harzianum* was prepared on PDA medium.

Bacterial bioagents, *P. fluorescens* and *B. subtilis* were prepared by inoculating a loopful culture in sterilized conical flask containing hundred ml nutrient broths. Broth culture was incubated at room temperature for five days. The two species of *Trichoderma* i.e. *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their antagonistic properties against wilt causing fungus by dual plate method.

Mycelial disc of 6 mm diameter cut from the margin of 5 days old cultures of both test pathogen and antagonists were placed opposite to each other on PDA in Petriplates (90mm). The distance between inoculum blocks was 6 cm. The petriplates with disc of *Fusarium* alone served as the control. The inoculated petriplates were incubated at $27\pm 2^{\circ}\text{C}$ in BOD incubator for 7 days. After the completion of incubation period the growth of *Fusarium oxysporum* f.sp. *ciceri* was measured and the per cent growth inhibition of intersecting colonies was calculated.

RESULT AND DISCUSSION

The data presented in Table 1 showed that highest per cent growth of inhibition of observed *Trichoderma harzianum* (76.66%) followed by *Bacillus subtilis* (63.14%). The lowest growth inhibition was observed in *Pseudomonas fluorescens* (53.52%). Similar finding by Deepashri and Raut (2005) studied the bio-control efficacy of antagonistic organism in managing the chickpea wilt and root rot pathogens. Twelve isolates of *Trichoderma* inhibited pathogen to varying degree. Maximum efficiency was recorded in APDRC (Tricho) 82.26% against *Fusarium oxysporum* f. sp. *ciceri*. Gupta *et al.* (2005) conducted field experiment to evaluate promising isolate of *Trichoderma viride* and *Rhizobium* as bio agents for controlling wilt complex fungi (*Fusarium*, *Sclerotium* and *Rhizoctonia*) and recorded higher grain yield of chickpea due to dual inoculation of *T. viride* and *Rhizobium*.

Table 1: Antagonistic effect of bio-agents on growth of *Fusarium oxysporum* f.sp. *ciceri*

Treatments	Bio agents	Mean colony diameter (mm)	Per cent growth inhibition
T1	<i>Bacillus subtilis</i>	33.17	63.14
T2	<i>Pseudomonas fluorescens</i>	41.83	53.52
T3	<i>Trichoderma harzianum</i>	21.00	76.66
T4	<i>Trichoderma viride</i>	24.50	72.77
T5	Control	90.00	0
'F test	Sig.		
SE(m) \pm	0.16		
CD (P=0.01)	0.74		

Pandey *et al.* (2005b) reported mechanism involved in antagonism behind *Trichoderma viride* might be biochemical and antibiosis effect rather than physical and chemical, examined the mode of parasitism between *Trichoderma*, *Fusarium* and *Rhizoctonia* under a microscope. Prameela *et al.* (2005) showed that *Trichoderma viride* and *Trichoderma harzianum* were maximum inhibition of 62% and 39% respectively against *Fusarium oxysporum* f.sp. *carthami* causing safflower wilt where as *Pseudomonas fluorescens* showed 36% inhibition. Rudresh *et al.* (2005) tested *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceri* cause wilt of chickpea and found that *T. harzianum* to inhibit the pathogen to a greater extent. Nikam *et al.* (2007) found that chemical seed treatment with Thiram (0.15%) + Carbendazim (0.1%) proved to be the most effective against *Fusarium oxysporum* f.sp. *ciceri*. *In vitro* evaluation of *Trichoderma* sp. against *F. oxysporum* f.sp. *ciceri* revealed the positive cumulative effect of *Trichoderma viride* + *Trichoderma harzianum* + *Trichoderma hamatum* in respect to the percent inhibition of the test fungus. The pot culture studies revealed that the soil application of *T. viride* (@ 25 kg/ha) as the most effective in reducing the incidence of chickpea wilt.

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