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In vitro Evaluation of *Trichoderma viride* and *Trichoderma harzianum* Against *Fusarium* Wilt of Chickpea

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ABSTRACT

Fusarium wilt (Fusarium oxysporum f. sp., ciceri) is one of the major yield limiting factors of chickpea (Cicer arietinum). For eco-friendly and sustainable management of the disease, two species of antagonists (Trichoderma virideand Trichoderma harzianum) and chemical fungicide (Carbendazim 50 WP) alone were evaluated against the pathogen. The study was carried out under laboratory conditions.Results showed that T. virideand T. harzianum alone or in combination significantly inhibited the mycelial growth of the pathogen. Different concentrations (10, 50 and 100 ppm) of Carbendazim 50 WP showed significant inhibition in the mycelia growth, and a concentration of 100 ppm completely inhibited the mycelia growth of the pathogen. Results of the study show that bio-agents significantly reduced the wilt incidence as compared to chemical fungicides.

Key words: *Chickpea, Fusarium oxysporum f. sp., ciceri, Trichoderma viride, Trichoderma harzianum, Carbendazim.*

INTRODUCTION

Pulses are important sources of protein for vegetarian population. Chickpea (*Cicer arietinum* L.) commonly known as gram is an important pulse crop. It is the world's fourth most important pulse crop after soybeans (*Glycine max* L.), beans (*Phaseolus vulgaris* L.) and peas (*Psium sativum*L.)[10]. In India, chickpea isranked first in terms of production and consumption in the world. About 65% of global area with 68% of global production of chickpea is contributed by India[1]. Low yield of chickpea is attributed to its susceptibility to several fungal, bacterial and viral diseases. *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo& K. Sato, is the most important soil-borne disease ofchickpea throughout the world and particularly in the Indian Subcontinent, the Mediterranean Basin and California[25]. At the national level, chickpea yield losses encounter due to wilt may vary between five to ten percent[9]. Since the pathogen is both seed and soil borne, drenching with fungicides is very expensive and impractical.

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Fusarium oxysporum f. sp. *Ciceri* is a facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years[14]. Therefore, integrated disease management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen pollution, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties[2]. In beneficial biological agent, *Trichoderma,* is a filamentous fungi which have attracted the attention because of their multi prong action against various plant pathogens[13]. Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma,* these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities[5]. *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular[11,17,22]. The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition[27]. Considering these points, the present study was conducted to find out the most effective species of *Trichoderma* and fungicide against chickpea *Fusarium* wilt.

MATERIALS AND METHODS

Fusarium oxysporum f. sp. c*iceri* was isolated from the infected roots of chickpea plants collected at *Fusarium* infested chickpea field in of chickpea growing area of eastern vidharbha of state Maharashtra, India. The fungus was cultivated on potato dextrose agar medium (PDA) and incubated for seven days at 25 ± 2°C (12/12 h light and dark cycle). The isolates were single spored and sub cultured onto PDA plates within a period of 2-3 months. Morphological characteristics of the fungal isolates were compared with standard descriptions given by Dasgupta (1988). Identification of *F. oxysporum* f. sp. c*iceri* isolates was done on the basis of cultural and morphological characteristics[20]. The fungal antagonist organisms *Trichoderma viride*and *Trichoderma harzianum* were also isolate from soils of eastern vidharbha. The efficacy of antagonists against the pathogen was initially evaluated on potato dextrose agar (PDA). Discs (5 mm diameter) of seven day old culture of bio-agents were inoculatedopposite to disc of the tested fungus (seven days old culture) in the same plate, both organisms were placed in such a manner that they would get equal opportunity for their growth[8]. The experiments were conducted with four replications\plates for each treatment, while control plates were inoculated only by tested fungus. Plates were then incubated at 27±1°C. Observation were recorded after seven days of inoculation including area covered by the *T. viride*and *T. harzianum* and the pathogen then percent of inhibition was calculated using the following formula[31]:

$$\text{Percent growth inhibition} = \frac{\text{Colony growth in control plate - Colony growth in intersecting plate}}{\text{Colony growth in control}} \times 100$$

Three concentrations of carbendazim (50 WP viz. 10, 50 and 100 ppm) were screened against the pathogen on PDA according to the poison food technique[24]. Four replications of each treatment along with control, maintained in completely randomized design, were incubated at 27°C. The radial growth of antagonist and pathogen was measured at 24 h intervals till 7th days and the percent inhibition was calculated by applying the above formula[31].

RESULTS AND DISCUSSION

The result presented in Table 1 indicated that the combined effect of both antagonists (*T. viride*+ *T. harzianum*) was found to be most

effective (86.57%) in inhibition of *Fusarium* mycelia growth as compared to the control followed significantly by *T. harzianum* (82.57%) and *T. viride*(80.24%) (Table 1). Several studies reported that inhibition of some soil borne pathogens, including *Fusarium oxysporum* f. sp. *ciceri* by *Trichoderma* species could probably be due to the secretion of extracellular cellwall degrading enzymes such as chitinase, β-1, 3-glucanase, β-1, 6-glucanase, protease, cellulease and lectin, which help mycoparasites to colonize their host [3,16,23,28]. Also, inhibition of the pathogen may be attributed to the production of secondary metabolites (such as glioviridin, viridin and gliotoxin) the antagonists[15]. Several studies reported that β-1-3 glucanase are the main skeletal polysac-charides of fungal cell wall and they also suggest chitinase and β-1-3 glucanase act as key enzymes in the lysis of phytopa-thogenic fungal cell wall during the antagonistic action of *Trichoderma*, hence fungal cell wall degrading enzymes of *Trichoderma* spp. are of special importance in plant defense mechanisms[18,19,29]. Some authors showed that microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front line for root against the pathogens[4]. Some scientists reported that *Trichoderma* species are almost found in all soils worldwide[21]. Through the population level, the bioagents are commonly found in the rhizosphere of chickpea plants required for effective disease management.

Table 1: In vitro evolution *Trichoderma* spp. and Carbendazim fungicide on mycelia growth of *F. oxysporum* f. sp. *ciceri*

Antagonist	**Percent of inhibition (%)**	**Carbendazim (50WP)**	**Percent of inhibition (%)**
Trichoderma viride	80.24	10 ppm	59.24
Trichoderma harzianum	82.57	50 ppm	72.57
Trichoderma viride + T. harzianum	86.57	100 ppm	81.46
Control	0.00 (90 mm radial growth)	Control	0.00
F test	S		S

Fungitoxic effect of different concentrations of carbendazim 50 WP on *Fusarium* organism was testedby applying poisoned food technique.Results showed different significant levels of fungitoxicity of the different concentrations of the fungicide against the pathogen (Table 1). The highest inhibition (81.46%) of the pathogen mycelia growth was recorded from 100 ppm concentration of the fungicide, followed by 50 (72.57%) and 10 ppm (59.24%) as compared to control (90 mm radial growth) (Table 1). Whereas other authors reported that carbendazim and thiram alone or in combination werehighly effective in inhibiting *in vitro* mycelia growth of the pathogen and in reducing wilt incidence under field condition[30]. Some authors found that the coating of chickpea seed with carbendazim was more effective in reducing wilt and increasing seed yield[7]. Some workers screened six fungicides against *F. oxysporum* f. sp. *ciceri in vitro* and reported that carbendazim was the most effective inhibitor when used at a rate of 100 mg/ml[12].

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