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COMPETENCE OF *TRICHODERMA VIRIDE* AS BIOCONTROL AGENT AGAINST SOIL BORNE *FUSARIUM OXYSPORUM* WILT DISEASE ON ONION CROP

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* which is the most important disease for onion while chemical applications dramatically decrease the production of the onion and generates impact to the soil environment. One of the major proficient techniques was to suppress various fungal diseases through biological control agents. Efficient *Trichoderma viride* and *Fusarium oxysporum* were isolated from Onion field soil and carried out a pot experiment for their biocontrol efficiency. The crop morphometric observation, biochemical characteristics, physico-chemical and biological profiles were analyzed for 30, 60 and 90 DAI rhizosphere soils. The combination of *Trichoderma viride* and *Fusarium oxysporum* showed increased level in all observed characteristics of crop and rhizosphere soil than individual one. Therefore, we conclude that this may be used as a universal biocontrol agent in agriculture.

Keywords: *Fusarium oxysporum*, *Trichoderma viride*, rhizosphere soil, fungal diseases, Morphometric, biological profiles.

INTRODUCTION

Fusarium oxysporum fungal pathogen produced soil borne wilt disease cause serious damage and losses to wide variety of crops, which hard to control. In that, onion is major cultivable economic vegetable crops in world, highly affected by fungal pathogens although; various management practices were implemented nevertheless very less effective because of the broad host range [1]. Especially methyl bromide widely used to manage soil borne fungal disease but soil fumigants were hazardous to the ecosystem [2]. Agricultural production for the past decades depends on the agrochemicals for the crop protection and their economic constancy. Moreover it causes negative effects later on use of microorganisms to control plant pathogens known as biocontrol; numerous biocontrol strategies proposed but their sensible application towards the soil are still limited.

The biocontrol through fungi have been applied to seeds and planting media to reduced soil borne wilt disease. Mainly *Trichoderma viride* free living fungi survived in soil and root ecosystem has the ability to control against phytopathogen fungi [3]. The main aim of the present work was to evaluate the efficiency of *Trichoderma viride* against *Fusarium oxysporum* on growth of the onion crop under nursery condition.

MATERIALS AND METHODS

Isolation of pathogenic and biocontrol fungi

Fusarium oxysporum as pathogens isolated from onion rhizosphere soil cultivated in Sirunatham village,

Thuraiyur taluk, Tiruchirappalli district and *Trichoderma viride* as a biocontrol agent from tomato rhizosphere soil Viralimalai village, Pudukottai district. These were microscopically identified based on microscopic characteristics. Isolates was maintained at 4ºC on Potato Dextrose Agar medium.

Antagonist activity of *F. oxysporum* against *T. viride*

The antagonist activity effectiveness was tested from *F. oxysporum* and *T. viride* cultured on PDA medium incubated for a week at 30ºC. In the pathogen, the antagonist fungal disc 0.3cm diameter colony was placed and reduction in mycelia growth of the pathogenic fungi [4].

Pot experiment

The onion bulb was sterilized using chlorination method [5]. Sterilized onion bulbs were undergone pot experiment under greenhouse conditions with sterilized soil. Triplicates were specified for each treatment in completely randomized block design method. The experimental treatments were followed as with control (T1), Pathogen (T2), Pathogen with biocontrol agent (T3), Pathogen with fungicide (T4), Pathogen, biocontrol agent and fungicide (T5). The crop morphometric characters like shoot length, number of leaves, internodes distance and root length were observed for every interval of 30 DAI until harvest with standard procedures.

Soil Profiles

Total fungal colonies in rhizosphere soil were enumerated by dilution plate method [6]; Macro nutrients nitrogen, phosphorous and potassium [7] and micronutrients such as copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) were estimated [8]. Protein [9], carbohydrate [10], phenol [11], chlorophyll [12] contents were also estimated from the leaves.

Statistical analysis

The mean and standard deviation for triplicate of each treatment was expressed as standard procedure by [13] was made using a SPSS 17.0 software package.

RESULT AND DISCUSSION

The antagonistic effect was tested *Trichoderma viride* against the test pathogen *Fusarium oxysporum* by *in vitro* dual culture experiment. In the present study the colony interaction of pathogen towards antagonists is maximum than the colony growth of pathogen away from the antagonist and growth of *Fusarium oxysporum* towards the centre of the plates in the absence of any antagonistic fungus (control) was 75 mm (r). Moreover, 81.6% of growth inhibition of pathogen in the zone of interaction (Fig.1). This represented that, it has an ability to control the soil borne wilt disease on onion crop. *Trichoderma* spp. secreted chitinase and B 1,3 glucanase in supernatants this was related to our report [14]. Also *T. viride* cell free culture filtrate inhibited the mycelia growth of *F. oxysporum* this was coinciding with our report [15].

The antagonistic effect of *Trichoderma* spp. may be due to more rapid mycelia growth than pathogenic fungi [16]. The inhibitory effect of these bioagents against tested pathogen was probably due to competition and antibiosis. *Trichoderma* species are most prominent antagonists, predominantly in the soil. Previous studies suggested that competition, antibiosis, as well as hyper-parasitic interactions were the mechanisms of antagonistic activity in biological control agents like *Trichoderma* [17].

A pot experiment has been carried out every 30 DAI to assess the efficiency of fungal strains against antagonist *F.oxysporum.* The total chlorophyll, carbohydrates and protein content were analysed and it showed that it gradually increased in their growth period from 30 to 90 DAI (Fig. 2). In overall period, the total chlorophyll (130.8mg/g), protein (42.06mg/g) and carbohydrate (51.26mg/g) were expressed maximum amount in T3 (pathogen with biocontrol agent) than all other treatments in 90 DAI Chlorophyll reduction in the other treatment may be due to changes in framework of chloroplast. Although, the reduction of chlorophyll, be the reason for decreased in the level of protein for other except T3 treatments.

Carbohydrates are the energy source for crops and also involved in physiological process like respiration, cell elongation which is essential for crop growth [18]. In our study, the total T3 carbohydrates increased may be attributed to increased level of chlorophyll and protein content because these translocate sugar molecules for the cell development. An aromatic compound of total phenol were analysed for every 30 days intervals consecutively.

In general, the phenol content was initially found to be maximum of 28.4mg/g in T3 treatment and next 60 and 90 DAI T2 treatment showed maximum content of 40.5 and 45.4 mg/ml respectively (Fig. 3). This may be due to infection of pathogen the phenol content was increased in T2 treatment. According, phytochemical studies the chlorophyll content directly proportional to carbohydrate and protein [19]. This was coinciding with our study that chlorophyll, carbohydrate and protein was increased in T3 and hence, clearly indicated that our biocontrol agent was potentially better biocontrol agent and further clearly proved that pathogen was controlled by biocontrol agent.

Soil physico-chemical characteristic were play an important role for plant growth. When the soils which around the roots were influenced through physically, chemically and biologically also root exudates were risk to the soil environment which affects growth of the plant [20]. The pH lies in its influence on availability of soil

nutrients, solubility of some toxic substances, break down of root cells and biological activities [21]. So, our present study reveals that pH is moderate alkaline from earlier period of the onion growth to till growth period ends at 8.2 – 8.5 range respectively. According to electrical conductivity all the samples gradually increased starting from 30 DAI and reach its maximum 0.82 ranges respectively. This may be due to the decrease amount of salinity in onion crop rhizosphere soil, because electrical conductivity related to soil salinity [22].

According to macro nutrients, mainly phosphorous persist in the soil in both organic and inorganic forms, but inorganic forms is more important for crop nutrition, if low phosphorous in soil indicate that acute deficiency in soil. But in our research, there are similarities in the relatively available phosphorous content for all the treatments but especially T2 (17.4-25.3 mg/ha) showed maximum in 30 and 90 DAI and fluctuation of high available nitrogen content (62-92.6mg/ha) in all soil samples respectively. While, available potassium content found to be maximum only T5 in 30DAI and later, it gradually decreased by increased in T2 treatments for other 60 and 90DAI respectively (Fig 4).

Whereas, in micronutrients content gradually increase until harvesting period, the copper and manganese showed maximum (1.70 & 5.97ug g^{-1}) in T3 treatment . While, iron content in T2 and zinc content in T1 treatment showed maximum in 90DAI (Fig. 5). It reveal that copper, manganese were inversely proportional to the iron and zinc content in the soil.

Figure 1. ***Fusarium oxysporum*** **Vs** ***Trichoderma viride***

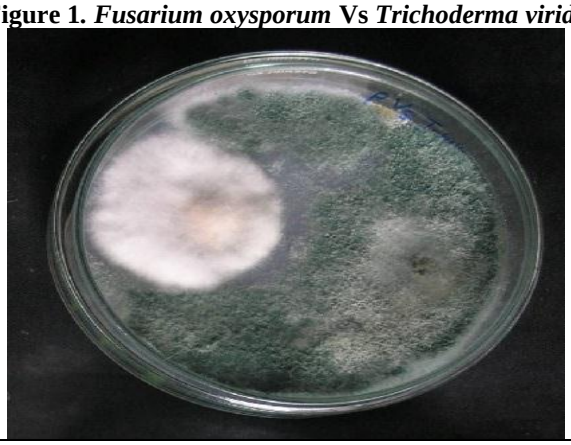


Figure 2. Chlorophyll content on 30, 60 and 90DAI for all treatments. Control (T1), Pathogen (T2), Pathogen with biocontrol agent (T3), Pathogen with fungicide (T4), Pathogen, biocontrol agent and fungicide (T5).

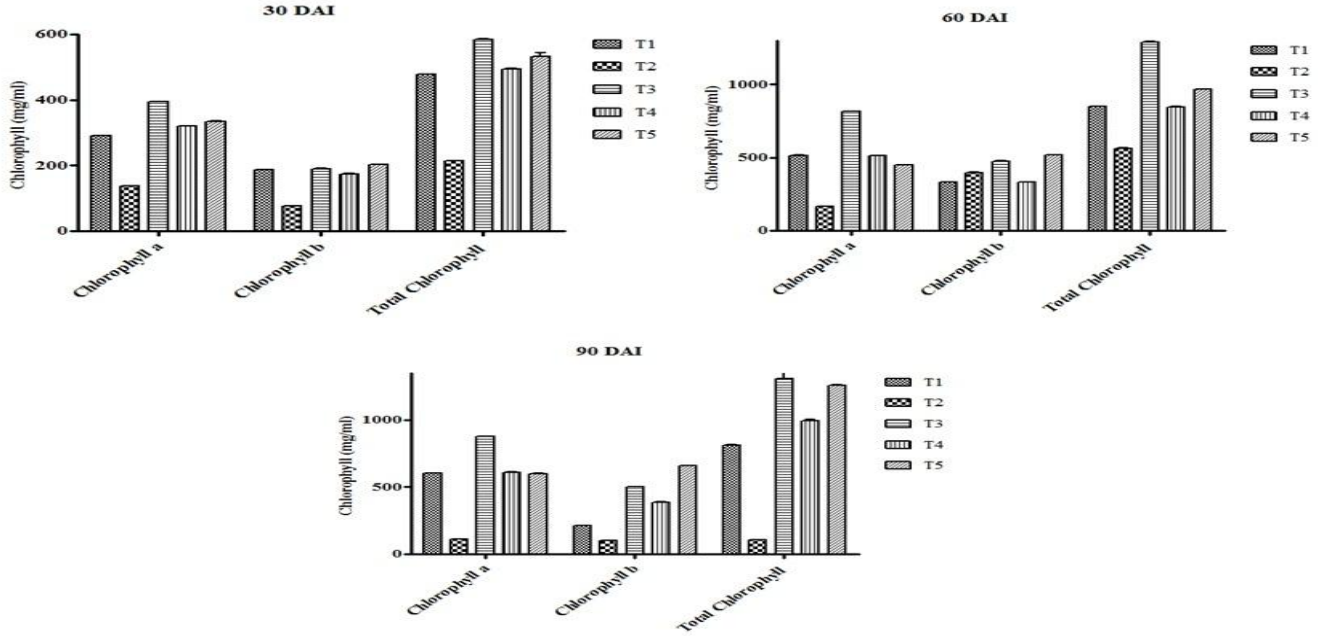


Figure 3. Phytochemical studies on 30, 60 and 90DAI for all treatments. Control (T1), Pathogen (T2), Pathogen with biocontrol agent (T3), Pathogen with fungicide (T4), Pathogen, biocontrol agent and fungicide (T5).

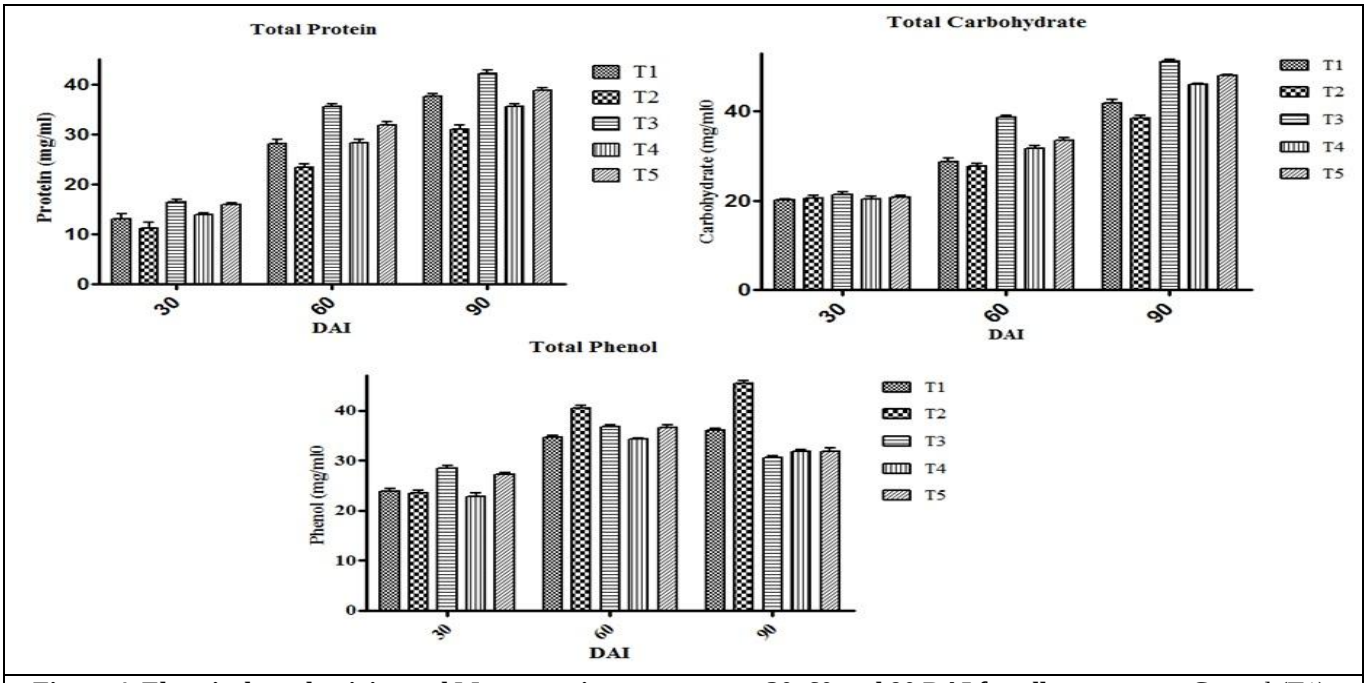


Figure 4. Electrical conductivity and Macronutrient contents on 30, 60 and 90 DAI for all treatments. Control (T1), Pathogen (T2), Pathogen with biocontrol agent (T3), Pathogen with fungicide (T4), Pathogen, biocontrol agent and fungicide (T5).

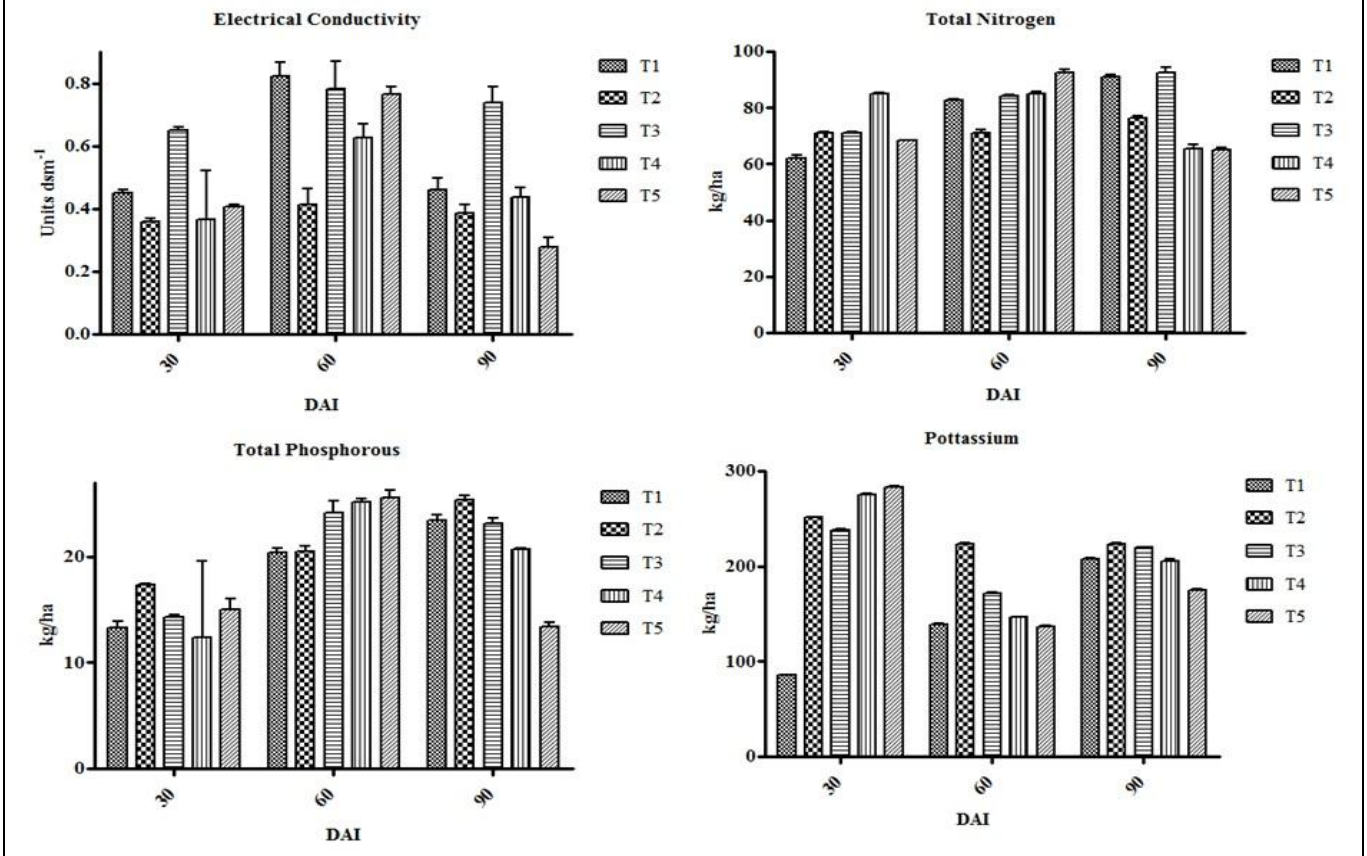
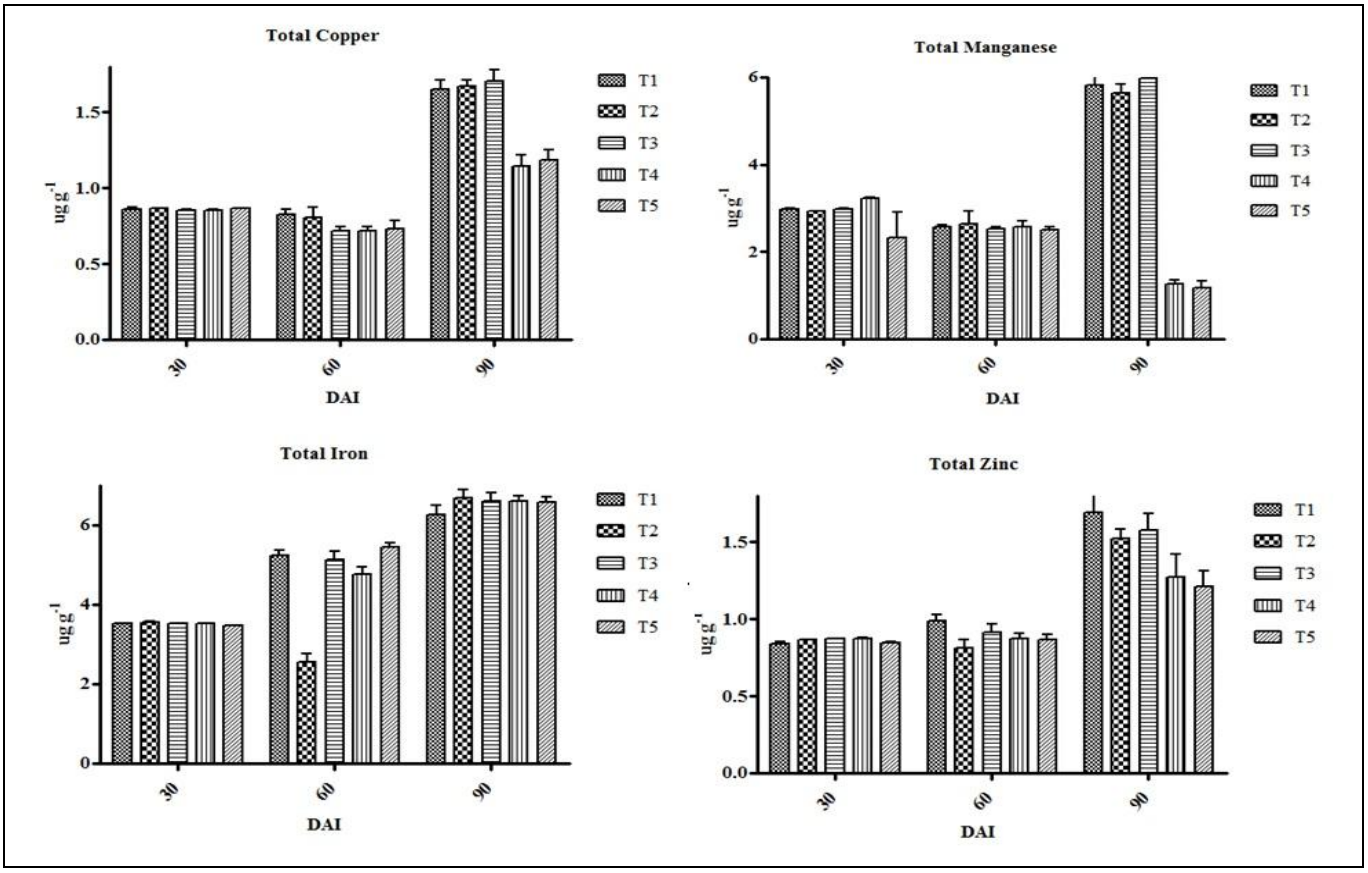


Figure 5. Micronutrient contents on 30, 60 and 90 DAI for all treatments. Control (T1), Pathogen (T2), Pathogen with biocontrol agent (T3), Pathogen with fungicide (T4), Pathogen, biocontrol agent and fungicide (T5).



CONCLUSION

The dual culture, plant morphometric and soil characteristics exhibited that T3 treatment soils were rich at their fertility and good at their plant growth, due to inhibit of the pathogen by biocontrol agent, which it clearly showed that our biocontrol agent was highly effective and it could have a potential ability to inhibit the pathogens and this can be used as a worldwide biocontrol agent in agriculture.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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