Research Article

Evaluation of Different Substrates for Development of *Trichoderma harzianum* Based Stock Cultures and Their Utilization in Management of Chilli Wilt Disease

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Abstract

In disease management, the indiscriminate use of chemicals have caused negative impact on environment and resulted in resistant strains of microbes to these chemicals. The chemical pesticides has undoubtly enhanced the food production, but has also adversely affected the environment and non-target beneficial organisms. Keeping in view these global problems, effective alternatives to chemical control is the need of hour. Biological control is one of the potential, cheap, ecofriendly and alternative approach for managing plant diseases and to combat the above said problems. Chilli (*Capsicum annuum L.*) one of the important commercial spices and solanaceous vegetable is encountered by many diseases and fungal wilt caused by *Fusarium* spp. has which is causing severe crop losses in chilli productivity throughout the world. *Trichoderma harzianum* and *Trichoderma viride* are the widely exploited species and have been used against about 70 soil borne plant pathogens. Looking towards need for cost effective production of ecofriendly biopesticide, present study was undertaken to evaluate different substrates like cereals, pulses and vermicompost, for development of stock cultures and fast biomass production of *Trichoderma harzianum* and their utilization in management of chilli wilt disease. The results revealed that the vermicompost followed by moong and maize having maximum colony forming unit are suitable for stock culture development and biomass production. The results also confirmed that the combination of all treatments followed by vermicompost is suitable for managing the chilli wilt when applied two weeks before transplanting in fields.

Keywords: Substrates, Trichoderma, Wilt, Disease, Chilli, Stock culture

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Introduction

Plant diseases are one of the major constraints in cultivation of crops worldwide, which results in loss of billions of dollars of farm produce annually. There is an utmost need to manage diseases and to make sure a steady and constant supply of marketable produce for the burgeoning world population [1]. In disease management, indiscriminate use of pesticides has resulted in accumulation of toxic compound potentially hazardous to human health and environment and also in the build up of resistance to the pathogens [2]. Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management strategies is essentially needed in present scenario [3]. In order to manage these problems, an effective alternatives approach to chemical control should be employed and considering the cost of chemical pesticides and hazards involved, biological control of plant diseases appears to be potential, non hazardous, viable, ecofriendly and alternative approach [4, 5]. Among the various microbes a few in fungi and bacteria have been systematically studied for their effective beneficial characteristics. Some *Trichoderma* spp. are acclaimed as very promising microbes against array of soil borne plant pathogenic fungi. *Trichoderma harzianum* and *Trichoderma viride* are the widely used species and have been exploited on about 87 different crops and about 70 soil borne plant pathogens [1]. Therefore cost effective, mass production, enhanced shelf life of formulation, establishment of bioagent in to targeted niche and consistency in disease management are the primary concern with augmentative biological control [6]. The National Farmer Policy (2007) has strongly recommended the promotion of biopesticide for increasing agricultural production, sustaining the health of farmers and environment [3].

Chilli (*Capsicum annuum* L.) is an important solanaceous vegetable cum commercial spice, having value addition in pharmaceuticals, cosmetics and beverages [7]. India produces 13.04 lakh tonne on an area of 7.94 lakh ha annually, and is major producer, exporter and consumer of chilli in world. The major chilli growing states are Andhra Pradesh, Gujarat, Karnataka, Odisha and Maharashtra [8]. Chilli is encountered by several fungal, bacterial and viral diseases such as damping off, leaf and fruit blight, anthracnose, leaf curls, little leaf and wilt are important and considerably

reduce the yields. Chilli wilt is one of the major diseases in India and yield loss to the tune of 50-80% was reported under heavy incidence [9]. Area under chilli is reducing due to wilt in the intermediate hill zone of Jammu and Kashmir [10] and has caused epiphytotics in several chilli growing areas in Kashmir valley, resulted in about 45-60 % yield losses [11]. However, application of fungicides under field conditions results in high cost, environment pollution and inconsistency in efficacy. Bio-control agents have been tested and successfully employed against soil borne pathogens including *Fusarium* [12]. Hence taking into consideration the importance of chilli and management of wilt disease using bioagent, our present study was undertaken to evaluate different substrates like cereals, pulses and vermicompost, for development of stock cultures for fast biomass production of *Trichoderma harzianum* and their utilization in management of wilt disease in chilli.

Materials and Methods

Isolation and Maintenance of Cultures

Trichoderma harzianum was isolated from the soils of apple orchard at ICAR-CITH, Srinagar by using potato dextrose agar (PDA) medium. Samples were inoculated over plates by serial dilution method and the plates were incubated at 26˚C for 4 days. The fungal colonies which were picked up and purified by streaking and incubated at 26˚C for 7-8 days [13]. As soon as the mycelial growth was visible in the PDA culture medium, the hyphal tips from the advancing mycelium were cut and transferred into the culture slants containing PDA medium for further purification and identification using morpho-cultural characters and available literature. Conidia forming fungal bodies were selected and microscopic observations were done [14]. One of the reference cultures with antipathogenic activity was provided by biocontrol Lab. division of plant pathology SKUAST-K Shalimar Srinagar.

Substrate Processing

Six substrates comprising of, maize, wheat, rice, moong, redgram and vermicompost were evaluated for estimating the biomass of *Trichoderma harzianum* at 26˚C. From grain one Kg substrates was well washed and kept overnight in distilled water to make them soft, excess water was drained off and 1 % sugar solution was added. Two hundred gram from all six substrates in 3 replications was packed separately in individual 500 ml conical flasks. These flasks were plugged with cotton and then autoclaved at 15 psi for 1hr at 121˚C consecutively for two days [14, 15].

Inoculation

All flasks were inoculated with 5 mm mycelial discs of 4-7 days old pre-cultured *T. harzianum* using a sterilized cork borer and incubated for 15 days at room temperature. After 15 days, biomass produced was estimated by weighing. Each treatment was replicated three times [16]. Enumerations of colony forming unit (CFU) of *T. harzianum* population were done at 10^{-8} dilution after 15 days after inoculation following serial dilution plate technique. To avoid clumping, on each day, the flasks were shaken vigorously to separate the culture and to break the mycelia mat [17].

Field evaluation

Stock Cultures were evaluated against chilli wilt under field conditions (Hot spot for wilt) at ICAR-CITH Srinagar. The experiment was conducted during the crop growing season from June to August during 2017. The variety chosen for the experiment is local popular and highly susceptible chilli variety Kashmiri long 1. Before 15 days of transplanting, stock cultures of all substrates were mixed with vermicompost in 1: 10 ratio and were added to experimental plots. Two irrigations were given at an interval of 15 days till seedlings were well established and developed, after that the frequent irrigations comprised of irrigating the plots after every 14 days interval [11]. Total six treatments comprising of stock culture on vermicompost (T1), market *Trichoderma* formulation (T2), stock culture on cereals (T3), stock culture on pulses(T4), stock cultures on cereals+ pulses+ vermicompost (T5) and control (T6) with each treatment replicated thrice in plot of size 1 m × 1m in RBD design. Observations on wilt were done at the flowering and fruit maturing stages of the crop and incidence percentage was calculated by using the formula as below.

$$\text{Percent Wilt Incidence} = \frac{\text{Number of Plants wilted}}{\text{Total Number of Plants Examined}} \times 100$$

Statistical Analysis

Statistical analysis was done using OPSTAT available on website http://hau.ernet.in/about/opstat.php [18].

Results and Discussion

Stock Cultures on Different Substrates

In the present study, fungus was confirmed as *Trichoderma harzianum* by morpho-cultural characters like colony colour, shape of conidiophore, phialides and conidia by using available literature [19] (**Figure 1**). Different substrates evaluated for stock culture development of *Trichoderma harzianum* were compared and the results of statistical analysis showed that the treatments T1, T5 and T6 are at par and there is a significant difference among the treatments. Among six substrates, vermicompost produced highest biomass followed by pulses and cereals, after two weeks (**Figure 2a-c**). Conidial quantity assessment revealed that after 15 days vermicompost followed by moong, produced highest colony forming units (CFU) at 10^{-8} dilution as shown in Fig 2c. The *Trichoderma* laden substrates after shade drying in laminar Airflow were grinded to powder form and stored at 4º C in small polythene bags (**Figure 3**) for further use. The fungus showed rapid multiplication on vermicompost as compared to other substrates evaluated during study. Soft texture and readily available nutrients in well decomposed vermicompost may have enhanced the growth of *T. harzianum*. Also the abundance of minerals in the pulses medium may have enhanced the growth of fungi.

Figure 1 Culture of *Trichoderma harzianum*

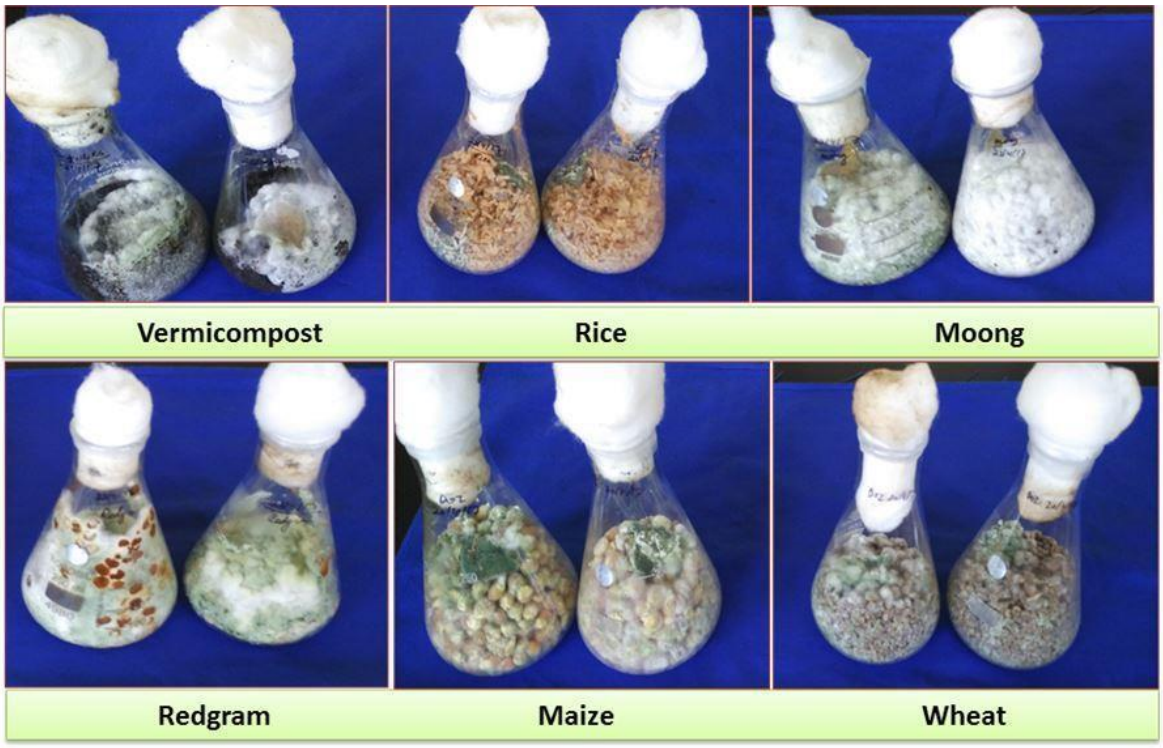

Figure 2a *Trichoderma harzianum* on Different Substrates

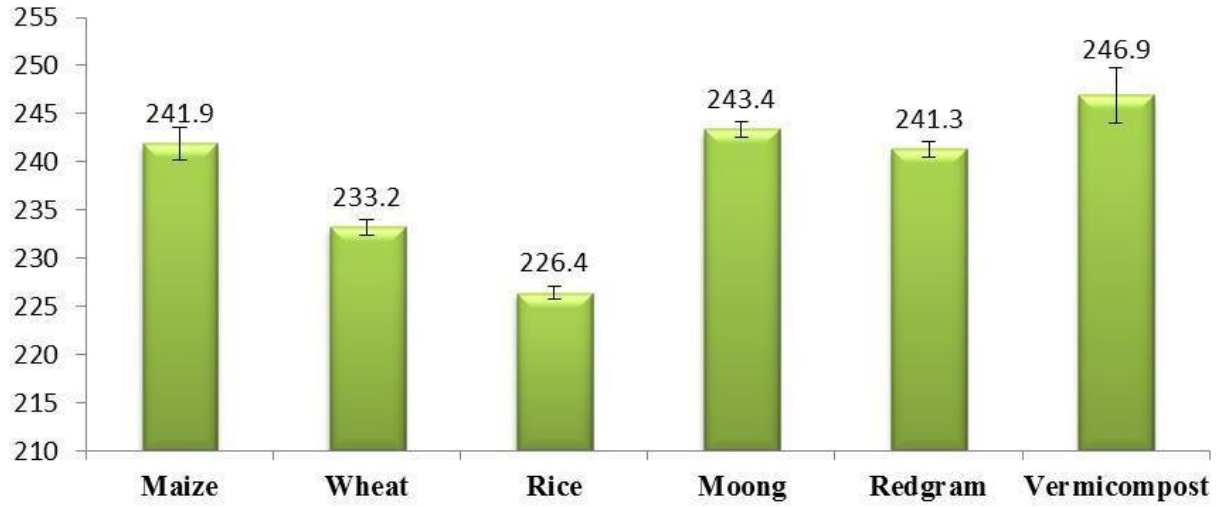

Figure 2b Average Weight (gm) of Biomass Produced on Different Substrates

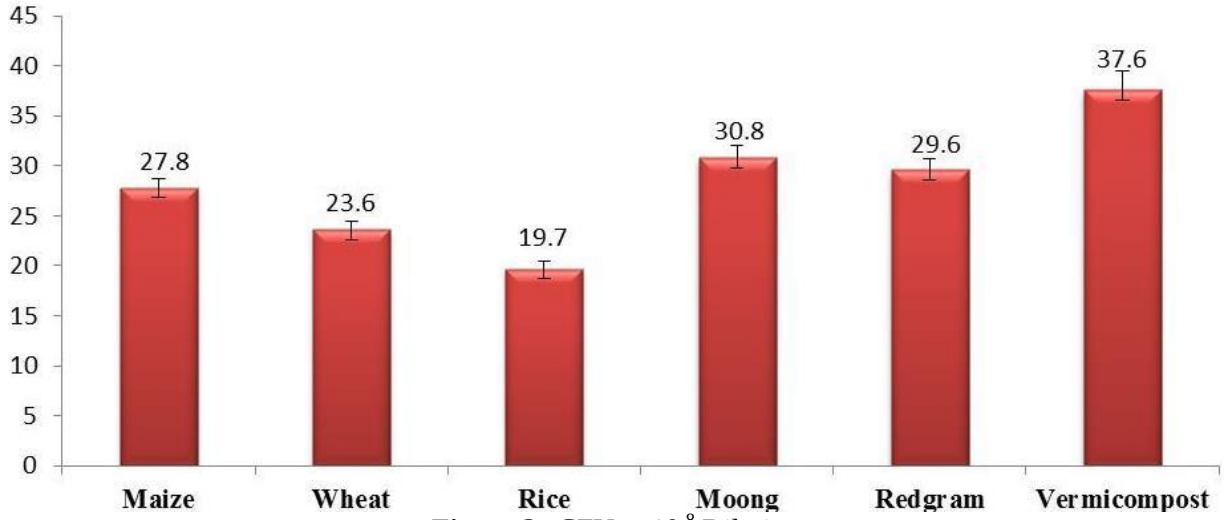

Figure 2c CFU at 10^{-8} Dilution

Figure 3 *T. harzianum* Stock cultures on Different Substrates

Management of Chilli wilt

All the six treatments evaluated whether individually or in combination significantly reduced chilli wilt, increased yields as well as other growth parameters (data not shown) as compared to control. The data shown in **Table 1** revealed that T5 reduces chilli wilt incidence significantly as compared to other treatments followed by T1, which may be due to large spore load of *T. harzianum* from all stock cultures with varied Colony forming units. Statistical analysis showed that treatments T1, T2, T3 and T4 were at par.

Table 1 Mean incidence of chilli wilt at different stages of crop using various treatments

Stock Cultures	Treatments	Mean % Incidence at Flowering	Mean % Incidence at Fruit Maturity
Vermicompost	T1	10.83	14.10
Market Trichoderma	T2	14.60	19.90
Cereals	T3	12.96	16.79
Pulses	T4	16.43	19.53
Vermicompost+Cereals+Pulses	T5	10.01	12.80
Control	T6	30.56	34.3
C.D.		8.70	9.60

In the present study vermicompost followed by pulses and cereals were found best for the growth of *Trichoderma harzianum*. Vermicompost shows higher CFU, as was earlier reported by Pan and Das [20], that vermicompost produced high population of *Trichoderma harzianum* out of four organic substrates evaluated. Suitability of the wheat for the growth of *Trichoderma* has been reported by Lewis [21, 22]. From this study it was clear that Trichoderma are able to grow on a wide variety of agriculture by products which can be useful to farmers to cultivate these fungi very easily. The higher CFU in vermicompost indicates it longer shelf- life as has also been reported earlier by other workers [20, 23]. The support of grain based substrates viz., maize toward higher multiplication of *Trichoderma* may be due higher presence of available nutrients. When these available nutrients are exhausted, the population of *Trichoderma* falls rapidly, but in vermicompost, nutrients are released slowly.

Our results confirmed that, *T. harzianum* gives encouraging results in managing the chilli wilt incidence. The results were also confirmed by other workers earlier in their separate studies on *Trichoderma harzianum* in management of chilli wilt disease [7, 11]. It has been proved that *Trichoderma* spp. inhibit pathogen invasion through phenomena of parasitism, antibiosis, competition, lysis of pathogenic hyphae and production of organic metabolites like chitinase and volatile inhibitory substance like acetaldehyde [24-28] and suggested several other possible mechanisms to explain this phenomenon including control of minor pathogens, production of plant hormones, production of vitamins, conversion of non-utilizable materials into a form that can be utilized by the plant and increased uptake and translocation of minerals, increases the efficiency of nutrient uptake solubilising certain insoluble nutrient elements like rock phosphate. The reduction of disease caused by *Fusarium* spp. may be due to higher chitinolytic activity by *Trichoderma* spp [7, 29]. It is evident from the results that vermicompost is not only a good substrate for multiplication and shelf-life maintenance of *T. harzianum* but also has the capacity to reduce disease incidence and improve plant growth parameters.

Conclusion

Chilli wilt is an economically important disease in Kashmir valley caused by *Fusarium* spp. Based on the results it is evident that vermicompost is not only a good substrate for multiplication and shelf-life maintenance of *T. harzianum* but also has the capacity to reduce disease incidence, improve plant growth parameters. Besides combination of all the treatments has significantly reduced the chilli wilt as compared to control.

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