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Studies on substrate evaluation for mass multiplication of *Trichoderma* spp. and their plant growth promotion activity in tomato

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ABSTRACT

The use of micro-organisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists and with additional benefit, when it provides the plant growth promotion activity. Ninety per cent of such applications have been carried out with different strains of *Trichoderma* which have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development. Due to their antifungal and plant growth promotion properties, many *Trichoderma* spp. like *T. asperellum, T. atroviride, T. harzianum, T. hamatum, T. koningii, T. virens* and *T. viride* are widely used for biocontrol of plant diseases incited by fungal pathogens. In the present investigation, a set of 5 local isolates of *Trichoderma harzianum* from Madhya Pradesh were used for evaluating their plant growth promotion potential in tomato and it was observed that isolates were having differential inborn capability to provide growth promotion when supplemented as seed and seedling treatment. With the aim of development of commercial formulation for direct use by farmers, five small millet substrates were used and it was observed that barnyard millet maximum supported the colonization of *Trichoderma* spp. and served as economic source for its multiplication to develop commercial formulation under laboratory conditions.

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INTRODUCTION

The genus *Trichoderma* is cosmopolitan in nature and survives on decaying wood and vegetative matter. Species of *Trichoderma* are frequently dominant components of the soil microflora in widely varying habitats. This may be attributed to the diverse metabolic capability of *Trichoderma* species and their aggressively competitive nature. *Trichoderma* species have been found to possess great potential in plant disease management (Kumar and Mukerji, 1996; Kumar *et al.*, 2010). Different *Trichoderma* species have been extensively tested as biocontrol agents against wide range of plant pathogens and several of them have been found potent against many soil and air-borne plant pathogenic fungi (Meki *et al.*, 2009). Antagonistic micro-organisms applied to seeds prior to planting colonize the rhizosphere of seedlings and thus are present at or near the pathogen's infection court where they act by competition, antibiosis, mycoparasitism, lysis and so on in the rhizosphere substrate (Harman, 1991). Different

species of *Trichoderma* are found in nature like *T. asperellum, T. atroviride, T. harzianum, T. hamatum, T. koningii, T. virens* and *T. viride* etc. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. *Trichoderma*, a filamentous soil inhabiting mycoparasite, is used in commercial preparation for biological control of many fungal plant pathogens (Jash, 2006). The mechanisms like antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solublization and sequestration of inorganic nutrients and inactivation of pathogen enzymes take place during various activities of *Trichoderma* spp. (Harman, 2000). Key feature of using compatible strains of plant growth promoting and biocontrol micro-organisms such as *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. etc., in a consortium is to maximize plant growth and biological control of phytopathogens has been globally demonstrated (Singh and Singh, 2012; Yobo *et al.*, 2009; Srivastava *et al.*, 2010).

Trichoderma species are known to proliferate abundantly in various natural soils and when added, establish intimate contact with a suitable organic food base (Lewis *et al.*, 1991). It has also been reported that solid-substrates promoted better growth and biological mechanism of *Trichoderma* spp. over the liquid substrates (Singh and Joshi, 2007). But, the large scale production of antagonistic micro-organisms is mostly made by solid-state fermentation using locally available agricultural waste materials like farm yard manure, oil cakes and compost. In addition to biocontrol properties, *Trichoderma* spp. are also economically important as sources of industrial enzymes. They produce several hydrolyzing enzymes of industrial significance like cellulases, hemi-cellulases and xylanases etc. Hence, they are important in the industry and can be used for large scale production of these enzymes for economy generation. *Trichoderma* spp. are most widely used biocontrol agents since they are reported to have antifungal, anti-nematodes, plant growth promoting and plant defense inducing activities. However, the major limitation of these biocontrol agents is not only their relatively short shelf-life but also inconsistent field performance (Zaidi and Singh, 2004). Among different approaches for mass multiplication at farmers' field, multiplication of *Trichoderma* on organic compost is one of the best approaches as it incorporates easily available and relatively inexpensive substrates like cow dung or FYM, vermicompost and poultry manure for the multiplication and delivery of *Trichoderma.* However, so far most of the research activities for mass multiplication of *Trichoderma* spp. were mainly based on its mass multiplication at farmer's field. But keeping the importance of this pathogen in mind, it is also important to identify a cheap and economic source of multiplication from which readymade formulation of *Trichoderma* can be prepared for direct use by farmers in their field. In this way, *Trichoderma* spp. are useful not only in biological control of plant pathogens but also provide better plant growth promotion activity.

There are various reports for plant growth promotion potential by *Trichoderma* but different isolates have variable capacity for growth promotion activity under field and laboratory conditions and it is of prime importance to evaluate their growth promotion potential before application in field to avoid inconsistence performance. Hence, there is a need to evaluate the potential of these micro-organisms for plant growth promotion activity under green house conditions before using them at field level. *Trichoderma* products or formulations will thus provide not only plant disease control but also give the local people opportunities to reduce health risks, costs and environmental damage due to over fungicide usages. Therefore, this study was undertaken with the objective of evaluating the potential of *Trichoderma* isolates from Madhya Pradesh for plant growth promotion activity in tomato and mass multiplication on different millet substrates.

MATERIAL AND METHODS

***Trichoderma harzianum* isolates :**

A set of five isolates of *T. harzianum* were procured from Department of Plant Pathology, College of Agriculture, Rewa and used in the present investigation. All of these isolates were isolated from Burhanpur, Rewa (Kuthulia), Khargone, Indore and Umaria locations of Madhya Pradesh and coded as T_1(BHN), T_2(RWA), T_3(KGN), T_4(IND) and T_5(UMR), respectively. The procured isolates of *T.harzianum* were maintained throughout the study by periodical transfers on Potato dextrose agar (PDA) medium. All the five isolates of *T. harzianum* have been shown in Fig. A.

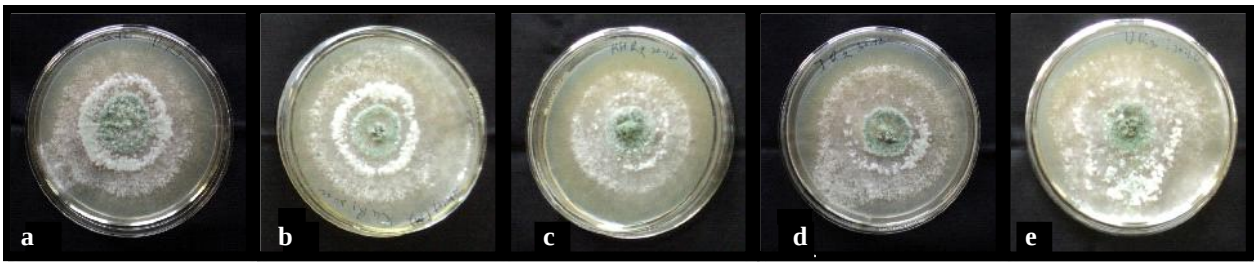

Fig. A : Different isolates of *Trichodermaharzianum* (a) T_1(BHN), (b) T_2(RWA), (c) T_3(KGN), (d) T_4(IND), (e) T_5(UMR)

Table A : Average colony forming unit (cfu)/g of *Trichoderma* on different millet substrates

Millet substrate	Scientific name	Common name	Variety	Avg. cfu/g x 106 of *Trichodemaa*
Barnyard millet	*Echinochloa frumentacea*	Saawan/Jhingora	Vl-172	15.75±0.96
Finger millet	*Eleusine coracana*	Ragi	GPU-28	12.75±0.96
Kodo millet	*Paspalum scrobiculatum*	Kodo	JK-155	10±0.82
Little millet	*Panicum sumatrense*	Kutki	JK-36	8.75±0.96
Foxtail millet	*Setaria italica*	Kakun	SIA-326	11.25±0.96
C.D. (P = 0.05)				1.59

[a] Mean of the four replicates with ±standard deviation

Substrate evaluation for mass multiplication of *Trichoderma* spp. :

Different types of minor millet substrates were evaluated for their ability to be colonized by *T. harzianum*. A set of 5 millet substrates (Barnyard millet-Saawan/Jhingoa, Finger millet-Ragi, Kodo millet-Kodo, Little millet-Kutki and Foxtail millet-Kakun) were used in the present investigation. Only one isolate of *T. harzianum* (T_2- RWA) was used for substrate evaluation in mass multiplication. For this purpose, 100 g of each millet substrate was taken in separate 250 ml flasks and soaked overnight in water. Next morning water was decanted and flasks were autoclaved. 10^5 conidia were inoculated in these 250 ml flasks and incubated at 27°C. After 15 days, the millet substrate was taken out and dried in shade. Further, this dried substrate was grinded and 1 g of the powder was diluted to 10^{-6} dilution and colony forming units (cfu) were counted on PDA plates. Details of variety used, scientific and common/local name of each millet substrate used in the present investigation has been provided in Table A.

Plant growth promotion activity in tomato :

Plant growth promotion activity of *T. harzianum* was studied under glass house conditions at College of Agriculture, Rewa. Seeds of the tomato cultivar Naveen were obtained from the Krishi Vigyan Kendra, Rewa (M.P.) and used in the present investigation. Tomato seeds were surface sterilized with 0.5 per cent sodium hypochlorite solution for 10 min. and then air dried. *Trichoderma* powder used for substrate evaluation in mass multiplication was then used in seed treatment of the tomato seed (@ 10 g/kg seed).The seeds were treated with different isolates of *T. harzianum*. Tomato seeds treated with different isolates of *Trichoderma* were sown in the plastic pots in four replications. Seeds were sown into plastic pots, each of 30 cm diameter and containing a soil mixture consisting of sand 3 kg/pot and 10 g slow-release fertiliser per kg (N:P:K 12:4:6). All pots were placed on a benchtop in greenhouse at 30 ± 5°C and six plants in each pot were maintained. Pots were irrigated on alternate days with equal amount of sterilized water. Soil surface occasionally stirred with plastic spatula to ensure good soil aeration. All the six plants were uprooted after 15 days of sowing for studying the growth potential of *Trichoderma* which was isolated from different locations of Madhya Pradesh. The roots of plants were dipped in conidial solution of respected cultures of *T.harzianum* before transferring them into another pot. After 10 days of transplanting, observations were recorded for no. of branches/plant, no. of leaves/plant, shoot length, root length, fresh weight and dry weight. For dry weight, all the plants were kept in hot air oven at 50°C. Dry weight of different replicates of each treatment was taken after every 24 hours until constant weight appeared.

Statistical analysis :

All the data were statistically analyzed at ARIS Cell, College of Agriculture, Rewa. Values from different experiments shown in tables were mean of data recorded with ± standard deviation (SD) of at least three determinations and analyzed by analysis of variance (ANOVA).The treatment means were compared with level of significance $p = 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Mass multiplication on different small millet substrates :

All of the five different small millet substrates, namely barnyard millet, finger millet, kodo millet, little millet and foxtail millet, tested for mass multiplication of *Trichoderma* well supported its growth on their surface. All the millet grains were fully covered with growth of *Trichoderma* and appeared with green colour conidial mass of *Trichoderma* after 15 days of incubation at 27°C. For evaluation of best substrate in mass multiplication, colony forming units were counted. It was observed from recorded data that all the substrates were maintaining different quantity of *Trichoderma* on their surface. However, maximum average colony forming unit of 15.75×10^6 was recorded in plating with dilution used from barnyard millet substrate treatment. This was followed by 12.75×10^6cfu from finger millet substrate treatment. Minimum cfu of 8.75×10^6 was recorded in plating with dilution used from little millet substrate. Detailed data for

cfu on each substrate has been provided in Table A. This can clearly depict that all the substrates were good source for mass multiplication of *Trichoderma*. However, barnyard millet substrate which is commonly known as saawan or jhingora in local language, maximum supported the colonization of *Trichoderma* and can be used as an economical source for its mass multiplication for various purposes under laboratory conditions.

Plant growth promotion activity in tomato :

All the isolates of *Trichoderma* were tested for their plant growth promotion activity in tomato (Table 1). Among all the tested isolates, *Trichoderma* isolate T_4(IND) showed maximum plant growth promotion in comparison to control and other tested isolates of *Trichoderma*. The maximum shoot and root length of 14.13±0.53 cm and 13.05±0.68 cm, respectively was recorded in plants treated with *Trichoderma* isolate T_4(IND). This was followed by isolate T_3(KGN) of *Trichoderma* where, respectively 13.23±0.53 cm and 12.18±0.57 cm shoot and root length was recorded. However, among five isolates of *Trichoderma*, minimum shoot and root length of 10.05±0.31 cm and 9.88±1.03 cm, respectively was recorded in treatment with *Trichoderma* isolate T_5(UMR). In control plants, where neither seed treatment nor seedling dip in *Trichoderma* was made, shoot and root length was recorded as 7.28±0.26 cm and 7.38±0.26 cm, respectively. Similarly, maximum no. of branches/plant and no. of leaves/ plant was recorded in treatment with *Trichoderma* isolate T_4(IND), which revealed the better capacity of this isolate to promote foliage development. Detailed data for growth attributes (shoot and root length, no. of branches/plant and no. of leaves/plants) for plants treated with respective isolates of *Trichoderma* has been given in Table 1. Pictorial representation for tomato plants treated with respective isolate of *Trichoderma* along with control plant has been given in Fig. 1.

Fig. 1 : Tomato seedlings treated with different isolates of *Trichoderma* (a) T_1(BHN), (b) T_2(RWA), (c) T_3(KGN, (d) T_4(IND), (e) T_5(UMR), (f) control

Plant biomass in form of fresh and dry weight of *Trichoderma* treated and control plants were also recorded (Table 2). The maximum fresh weight of 10.83±0.64 g was recorded in plants treated with *Trichoderma* isolate T_4(IND). However, minimum fresh weight of 7.63±0.58 g was recorded in plants treated with *Trichoderma* isolate T_5(UMR). In control/untreated plants 7.08±0.62 g fresh weight was recorded. Dry weight of tomato plants was also recorded for the same plants after drying in hot air oven and maximum

Table 1 : Effect of different isolates of *Trichoderma harzianum* on growth attributes of tomato plant

Isolate	Root length (cm)[a]	Shoot length (cm)[a]	No. of branches/plant[a]	No. of leaves/plant[a]
T_1(BHN)	11.23±0.57	13.08±0.43	6.00±0.82	15.75±2.06
T_2(RWA)	11.05±0.74	12.85±0.82	5.75±0.50	15.50±0.58
T_3(KGN)	12.18±0.57	13.23±0.53	6.50±0.58	16.25±0.96
T_4(IND)	13.05±0.68	14.13±0.53	7.50±0.58	18.50±1.29
T_5(UMR)	9.88±1.03	10.05±0.31	5.25±0.50	14.25±1.26
Control	7.38±0.26	7.28±0.26	5.00±0.82	12.00±0.82
C.D. (P = 0.05)	1.13	0.85	1.07	2.07

[a] Mean of the four replicates with ±standard deviation

Table 2 : Effect of different isolates of *Trichoderma* on biomass of tomato plant

Isolate	Fresh weight (g) [a]	Dry weight (g) [a]	S/R ratio
T_1(BHN)	9.96±0.35	2.99±0.39	1.16
T_2(RWA)	8.83±0.77	2.69±0.26	1.16
T_3(KGN)	10.38±0.31	3.04±0.34	1.09
T_4(IND)	10.83±0.64	3.19±0.44	1.08
T_5(UMR)	7.63±0.58	2.55±0.30	1.02
Control	7.08±0.62	2.42±0.33	0.99
C.D. (P=0.05)	0.94	0.57	

[a] Mean of the four replicates with ±standard deviation

dry weight of 3.19±0.44 g was recorded in plants treated with *Trichoderma* isolate T_4(IND). However, in plants treated with T_5(UMR), minimum dry weight of 2.55±0.30 g was recorded. In control plants 2.42±0.33 g dry weight was recorded. Ratio (S/R) of above ground part (shoot length) and below ground part (root length) was calculated and it was observed that all the isolates of *Trichoderma* significantly increased the ratio of shoot and root length. In all the treatments except control, the S/R ratio was greater than one, which shows the more increase in shoot length by these treatments. The maximum S/R ratio of 1.16 was observed in treatment with *Trichoderma* isolate T_1(BHN) and T_2(RWA). In control plants, where *Trichoderma* was not used in any practice, minimum S/R ratio of 0.99 was calculated. The S/R ratio was recorded less in treatment with T_4(IND) in comparison to T_1(BHN), T_2(RWA) and T_3(KGN) because of more enhancement in both root and shoot length. This can also be observed by per cent increase in growth of shoot and root by treatment of different isolates of *Trichoderma*. Per cent increase in growth for different growth parameters was calculated over control by treatment of different isolates of *Trichoderma* and it was observed that maximum increase was arisen in treatment with T_4(IND) isolate for all the recorded parameters. Among different isolates, T_4(IND) isolate increased 94.23 per cent and 76.95 per cent shoot and root length, respectively in comparison to control. However, respectively 53.02 per cent and 31.82 per cent fresh and dry weight of plants was increased. This showed the better potential of this isolate for plant growth promotion. Hence, isolates of *T. harzianum* showed differential behaviour in their plant growth promotion activity. Fig. 2 represents the per cent increase in different growth parameters *viz.*, root length, shoot length, fresh and dry weight of plant after treatment with different isolates of *Trichoderma* in comparison to control.

With the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, several *Trichoderma* species have been formulated for commercial production pertaining to protection and growth enhancement of a number of crops in several countries. There are various reports of plant growth promotion activity using micro-organisms but capability of *Trichoderma* spp. varies among different isolates when evaluated for their growth potential and thus results in inconsistence field performance. Khan *et al*. (2005) reported that *T.harzianum*, examined for its effects on emergence and vigour of rice seedlings through seed or soil treatments, all doses of *T.harzianum*, in both the experiments significantly increased seedling emergence, root and shoot length, fresh and dry weight of root of rice seedlings, as compared to check. Ha (2010) conducted his experiments on several crops such as: peanut, tomato, cucumber and durian and concluded that crop treated with *Trichoderma* grew better and had higher

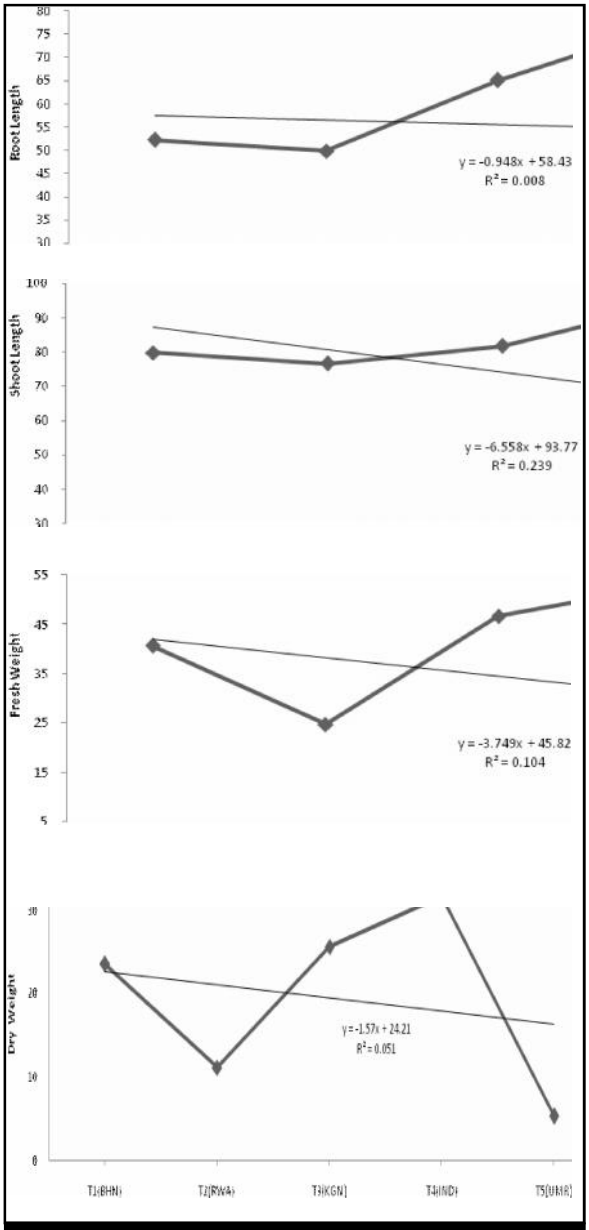

Fig. 2 : Per cent increase in growth for different parameters by isolates of T. *harzianum*

yields as compared to the one without application. John *et al*. (2010) reported that *Trichoderma* showed growth promoting action on soybean. *Trichoderma* enhanced growth of shoot and root systems and fruit yield after 12 weeks of growth. *Pythium* and *Fusarium* infected plants treated with *Trichoderma* had ~194 per cent and 141per cent more height than pathogens alone. In the present investigation also, respectively 94.23 per cent and 76.95 per cent increase in shoot and root length was observed when seeds were treated with T_4(IND) isolate of *Trichoderma*. These results clearly depict the variability among the isolates of *Trichoderma*. In

the present results are in agreement with Singh *et al*. (1995) as they also demonstrated that variability exists between the isolates of same species of *Trichoderma*. As we can see in the present investigation and other studies also, there was significant increase in root length of plant treated with *Trichoderma* spp. which might be due to better capacity of longer root system which augments uptake of nutrients and water from the soil. Due to more shoot length and presence of more number of leaves, plants look healthy and have better potential to fight against several stress conditions present in their vicinity.

For mass multiplication of *Trichoderma* spp., Prakas *et al*. (1997) evaluated four substrates (neem cake, farm yard manure, coffee husk and tea waste) and concluded that tea waste was the best medium. The cultures, mass multiplied in tea waste could also be stored for 3 months without much reduction in the population of biocontrol agents. Zaidi and Singh (2004) conducted their study on development of improved technology for mass multiplication and delivery of fungal (*Trichoderma*) and bacterial (*Pseudomonas*) biocontrol agent. They concluded that fresh cow dung, FYM, and poultry manure supported excellent growth of both *T. harzianum* and *Pseudomonas fluorescence*. Population of *T. harzianum* on colonized cow dung, FYM, and poultry manure may be as high as 10^8 cfu g^{-1} air dried compost. Also press mud, by-product of sugar industry, colonized well by *T. harzianum*. Similarly Sangle and Bambawale (2005), Singh and Joshi (2007), and Parab *et al*. (2008) used different substrates for mass multiplication and reported about the quantity of colonized *Trichoderma* spp. in the form of cfu/g of powder on different substrates for mass multiplication.

On the other hand, all of these studies were mainly confined to mass multiplication of *Trichoderma* spp. at farmers' field whereas from present investigation, out of five millet substrates, barnyard millet maximum supported the colonization of *Trichoderma* with adequate cfu on its surface and can be used as an economical source for various industrial purposes and development of ready to use formulation for the benefit of farmers. In this way, results of the present study agree with the previous results in quantity of cfu but with different substrates which can be used under laboratory conditions. This is a preliminary step towards exploring the indigenous diversity of *Trichoderma* in their plant growth promotion potential properties from M.P. The present study threw light towards the aspects of growth enhancement of tomato plants when treated with isolates of *Trichoderma* spp. over control. Further, studies are required to verify these results under field conditions together with different carrier to the biocontrol agent so that this can contribute to minimise the risks and hazards of toxic fungicides and provide plant growth promotion.

Conclusion :

Barnyard millet is a cost-effective, easily available source for mass multiplication of *Trichoderma* spp. Different isolates of *Trichoderma* exhibit differential behavior in their plant growth promotion activity.

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