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Survival and Population Dynamics of *Trichoderma* sp. Under Field Conditions at Mangalpur, Chitwan

Pratishtha Adhikari[1*] and Deepika Timsina[2]
[1] Department of Plant Pathology
[2] Department of Agronomy
Agriculture and Forestry University, Nepal
[*]Corresponding author's email: padhikari@afu.edu.np

Abstract

The antagonist fungi *Trichoderma* are effective bio-control agents against various soil-borne plant pathogens. *Trichoderma* species thrive well in soil and decompose organic matter. A high soil population is necessary for pathogen control. However, limited information exists on how different crop residues influence *Trichoderma* proliferation and survival in field conditions. An experiment was conducted aiming to study the survival and population dynamics of *Trichoderma* on various crop residues. *Trichoderma* sp. was isolated from agricultural land and mass-cultured on a rice husk-rice bran mixture. Field plots were established in a randomized complete block design with different crop residues (rice, wheat, maize, soybean, finger millet, lentil, and bagasse) in June 2021. *Trichoderma* inoculum was applied on fallow land, and soil samples were collected monthly for six months to assess the fungal population. Results revealed significant effects ($P<0.05$) of crop residues on *Trichoderma* populations. Rice straw supported the highest population densities over six months period. Over time, populations declined, with significant differences observed between treatments. The study underscores the importance of crop residue management in maintaining soil *Trichoderma* populations for optimizing their use as biocontrol agents.

Keywords: Bagasse, crop residue, mass culture, population density, rice straw, *Trichoderma*

Introduction

The genus *Trichoderma* is anamorphic fungi growing in soil and decomposing organic matter are the promising antagonistic fungi, established in agriculture use (Rey et al., 2004). They show antagonistic activity under both *in vitro* and *in vivo* conditions by competing for nutrients and space, antibiosis, mycoparasitism, promoting plant growth and plant defense responses (Brotman et al., 2010). These fungi can adapt to diverse environmental conditions by regulating their growth, sporulation, and enzyme production ability. *Trichoderma* species are successfully used as bio-control agents against a wide range of soil-borne plant pathogens. However, for the effective management of soil-borne pathogens, a high population of the antagonists is required in the soil (Nakkeeran et al., 2016). One of the desirable characteristics of a successful antagonist is considered to be its ability to grow and proliferate excessively in soil (Baker & Cook, 1974). Production and application of bio-control agents on large scale and their survival in the field are among the major limitation of their successful use in agriculture. Exotic *Trichoderma* when applied to the soil, cannot survive for long period without the food base (Lewis & Papavizas, 1984). *Trichoderma* species are affected by soil pH, moisture and electrical conductivity (Mayo-Prieto et al., 2021). According to Wong et al. (2002), moist soil conditions is favorable for the activity and growth of *Trichoderma koningii*. Similarly, *Trichoderma* spp. are favored by the acidic soil condition. Singh et al. (1998) reported antagonistic ability of *Trichoderma* increased with high soil moisture level. According to Timper (2011), certain soil type and texture support growth of specific kind of *Trichoderma*. Amir-Ahmadi et al. (2017) reported the best performance of *T. harzianum* in sandy loam soil and loam soil containing 2% organic matter. Soil texture affect the ability of antagonistic fungi to suppress the plant pathogens (Moosavi & Zare, 2015).

Appropriate formulation, effective delivery, and viability of inoculum during delivery are important for biological control using antagonists. Multiplication of *Trichoderma* in easily available and cheap crop byproducts with long shelf life would be effective for field application (Thangavelu et al., 2004). Solid substrates stimulate the natural conditions for the multiplication of *Trichoderma*. Also, these substrates enhance sporulation with longer viability (Naeimi et al., 2020). *Trichoderma* spp. are highly efficient colonizers of the substrate they grow on. There are numerous reports regarding the growth and colonization of various organic substrates used as the mass culture for *Trichoderma*. These fungi have been successfully grown on solid substrates like sorghum grain, maize seed, wheat seed, wheat straw, wheat bran, wheat bran-saw dust, vegetable and fruit wastes, compost and poultry manure, sugarcane bagasse etc. for their mass multiplication (Babu & Pallavi, 2013; Mohidin et al., 2017; Simon, 2011; Subash et al., 2014). Mohidin et al. (2017); Babu and Pallavi (2013) found maximum shelf-life in maize and wheat seeds supplemented with molasses. Similarly, Subash et al. (2014)

reported good sporulation and shelf life of *Trichoderma* spp. in sorghum grains. However, there is little information available concerning the effect of different crop residues on the proliferation and survival of *Trichoderma* in field conditions. Lewis & Papavizas (1984) reported a steady decline in population densities of *Trichoderma* in soil. Gangwar et al. (2013) reported a significant effect of different substrates on the population of *Trichoderma* spp. This study aims to understand the survival and population dynamics of *Trichoderma* in tropical field conditions.

Materials and Method

Isolation of *Trichoderma*

Trichoderma strain was isolated from agricultural soil by using Trichoderma selective medium (TSM). The ingredients used in the medium were $MgSO_4.7H_2O$ (0.2g), K_2HPO_4 (0.9g), KCl (0.15g), NH_4NO_3 (1.0g), Glucose (3.0g), Agar-agar (20g), Distilled water (1 liter), Streptomycin sulphate (0.02g), Captan (0.2g) and Rose Bengal (0.15g). The plates were incubated at 25ºC and examined daily for the growth of *Trichoderma* colonies for 5 days. The entire process was carried out under laminar flow station. Appearance of *Trichoderma* colonies were observed on 5th day of inoculation. Each colony that appeared in the TSM plate were transferred to PDA tubes. Mass culture of the *Trichoderma* sp. was prepared in sterilized mixture of rice husk and rice bran at 1:5 ratio. Rice husk was soaked in water for 10–12 h and rinsed in running tap water until the water ran clear. The excess water was completely drained and rice bran was mixed with rice husk and transferred to polypropylene bags and autoclaved at 121°C for 15 minutes for two consecutive days. After cooling, the mixture was aseptically inoculated with *Trichoderma* sp. grown on PDA media was cut using a sterile cork borer (5 mm diameter), five plugs were transferred into the mixture of rice husk and rice bran and incubated for two weeks.

Survival of Trichoderma in Crop Rresidue

Establishing Crop Residue Treatments in the Field Plots

Field plots with an area of 1 sqm with a 0.5 m alley between plots were established in a randomized complete block design at Mangalpur, Chitwan, Nepal in June 2020. This study was carried out in fallow land to minimize the effect of previous crop residue on survival of *Trichoderma* in the field. Crop residue of rice, wheat, maize, soybean, Finger mille, Lentil, and bagasse were used for the study. Crop residue was manually spread at the rate of 2 kg per plot. Immediately after the application of crop residue, the mass culture of *Trichoderma* sp. was broadcasted manually on all the plots, as uniformly as possible, at a rate of 25 g/plot. After spreading the residue and the inoculum, the plots were carefully tilled. Care was taken to minimize the movement or spill of the residue and the inoculum into adjacent plots and or beyond the alleys between the plots. The plots

were initially irrigated to maintain soil moisture. Plots were maintained weed-free by manual weeding.

Soil Sampling from the Plot

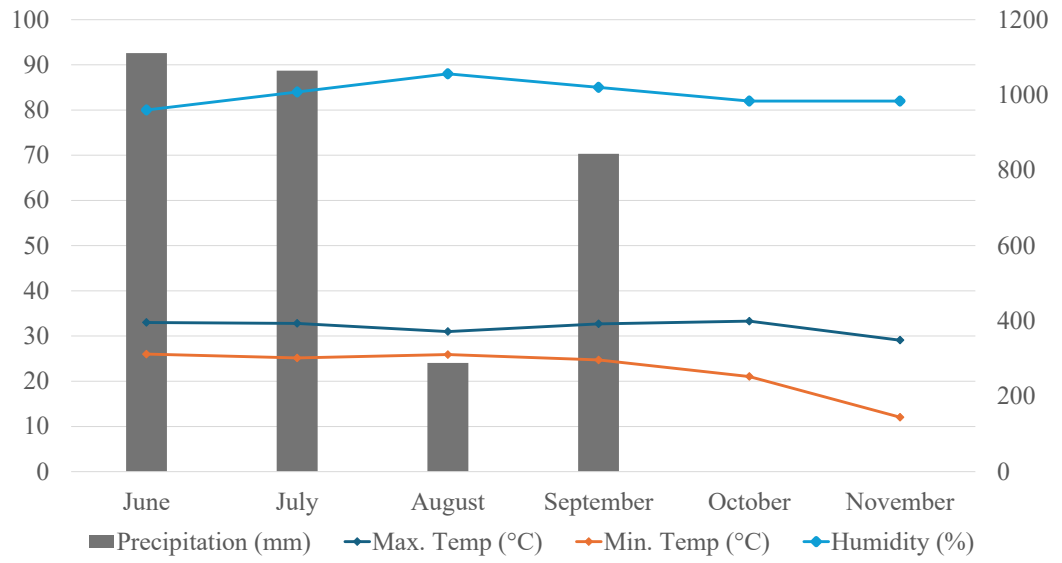
For assessing the population of *Trichoderma* in soil, soil was sampled from all plots every month for six-months period. Soil was collected from a depth of 4 to 6 cm in all plots. The samples were taken from five arbitrarily selected spots in each plot and were bulked and collected in plastic bags.

Isolation and Enumeration andCconfirmation of Trichoderma sp. from Soil

One g of soil was weighed and transferred to a test tube containing nine ml distilled water and agitated for five minutes. The soil solution was diluted by serially dilution method to 10^{-3}. One ml aliquot was pipetted out from each test tube and transferred into a test tube containing nine ml distilled water for three fold dilutions. From each dilution, 10 µl were pipetted out and transferred onto a nine cm Petri dish containing TSM. Soil suspension on TSM plates was spread using a glass rod. Petri dishes were incubated in an incubator for four days at 25 ± 1°C. *Trichoderma* sp. population was assessed by enumerating colonies as colony-forming units per gram of soil (CFU/g of soil). Confirmation of *Trichoderma* sp. from the field was carried out by morphological study.

Figure 1

The weather condition of Mangalpur, Chitwan during the research period 2020 (source: NMRP, Rampur, Chitwan)



Statistical Analysis

The data recorded were tabulated in Microsoft Excel and analyzed using R-studio version 4.1.0. Treatment mean was compared using Duncan's multiple range test (DMRT) at 1 and 5% levels of significance. Count data were subjected to square root transformation. Graphs were prepared by using MS Excel.

Results and Discussion

Effect of Crop Residue on *Trichoderma* Populations in Soil

The population of *Trichoderma* was influenced significantly (P<0.05) by applied crop residue. *Trichoderma* population was significantly higher in rice straw as compared to all other treatments in six-month period (Table 1). In the first month, the highest *Trichoderma*, colony forming unit (CFU)/g soil was recorded in rice straw (71.33×10^5) which was at par with soybean stalk (52.33×10^5). The lowest *Trichoderma* population was observed in lentil stover (19.66×10^5) which was less than the control (26.33×10^5) plots. For the first month, plots amended with Finger millet husk, Wheat straw, Bagasse, and Maize stover did not differ significantly for *Trichoderma* population. In second month, the CFU/g soil of *Trichoderma* was significantly higher in plot amended with rice straw (42.33×10^5) compared with all other treatments and lowest in control plot (14.00×10^5). In the third month, CFU of *Trichoderma* was highest in rice straw (24.67×10^5) followed by bagasse (16.67×10^5) and wheat straw (16.33×10^5) and lowest in control plot (7.00×10^3). While in fourth and fifth months, plots amended with crop residues did not differ significantly for the population of *Trichoderma* except for control plot. However, in sixth month *Trichoderma* population was highest in rice straw (10.00×10^3) followed by soybean stalk (9.00×10^3), wheat straw (8.33×10^3) and bagasse (8.00×10^3) and lowest in control plot (4.00×10^2). Population of *Trichoderma* reduced with increase in days after inoculation in all crop residues. While it was increased in soybean stalk from 9.67×10^4 to 10.67×10^4 CFU/g soil in 120 days after inoculation.

Table 1

Effect of crop residues on survival of Trichoderma isolate, Mangalpur, Chitwan 2020

Crop residue	CFU/g soil (days after inoculation)					
	30	60	90	120	150	180
Rice straw	71.33×10^{5a} (6.85)	42.33×10^{5a} (6.62)	24.67×10^{4a} (5.29)	13.67×10^{4a} (4.73)	11.00×10^{3a} (4.03)	$10.00 \times 10^{3\ a}$ (3.99)
Soybean stalk	$52.33 \times 10^{5\ ab}$ (6.70)	$22.66 \times 10^{5\ b}$ (5.82)	$9.67 \times 10^{4\ bc}$ (4.77)	$10.67 \times 10^{4\ a}$ (4.66)	$10.00 \times 10^{3\ b}$ (3.99)	$9.00 \times 10^{3\ a}$ (3.93)

Finger millet husk	44.66 × $10^{5\ bc}$ (6.54)	24.66 × $10^{5\ ab}$ (6.28)	14.67 × $10^{4\ ab}$ (5.01)	10.00 × $10^{4\ a}$ (4.68)	5.33 × $10^{3\ b}$ (3.72)	5.00 × $10^{3\ b}$ (3.69)
Wheat straw	30.33 × $10^{5\ bc}$ (6.31)	24.00 × $10^{5\ a}$ (6.45)	16.33 × $10^{4\ ab}$ (5.05)	13.00 × $10^{4\ a}$ (4.66)	10.66 × $10^{3\ a}$ (4.00)	8.33 × $10^{3\ ab}$ (3.99)
Bagasse	29.33 × $10^{5\ bc}$ (6.45)	20.00 × $10^{5\ b}$ (5.66)	16.67 × $10^{4\ bc}$ (4.25)	11.67 × $10^{4\ a}$ (4.62)	10.66 × $10^{3\ a}$ (4.01)	8.00 × $10^{3\ ab}$ (3.89)
Maize stover	29.33 × $10^{5\ bc}$ (6.45)	17.33 × $10^{5\ ab}$ (6.21)	13.67 × $10^{4\ ab}$ (4.82)	11.33 × $10^{4\ a}$ (4.69)	10.33 × $10^{3\ a}$ (3.99)	7.66 × $10^{3\ ab}$ (3.87)
Lentil stover	19.66 × $10^{5\ c}$ (6.09)	15.67 × $10^{5\ ab}$ (6.13)	11.33 × $10^{4\ bc}$ (4.66)	10.00 × $10^{4\ a}$ (4.66)	8.33 × $10^{3\ ab}$ (3.98)	7.66 ×$10^{3\ ab}$ (3.88)
Control	26.33 × $10^{5\ bc}$ (6.41)	14.00 × $10^{5\ b}$ (5.77)	7.00 × $10^{3\ c}$ (3.99)	4.33 × $10^{3\ b}$ (3.55)	4.00 × $10^{2\ c}$ (2.59)	4.00 × $10^{2\ c}$ (2.55)
F- test	*	*	*	***	***	***
LSD (=0.05)	554.83	462.48	104.67	90.45	25.34	20.48
CV (%)	3.52	5.39	9.29	1.14	3.53	3.26

Note. Figures followed by the same letter in the column are not significantly different by DMRT. Figures in the parentheses are square root transformation values.

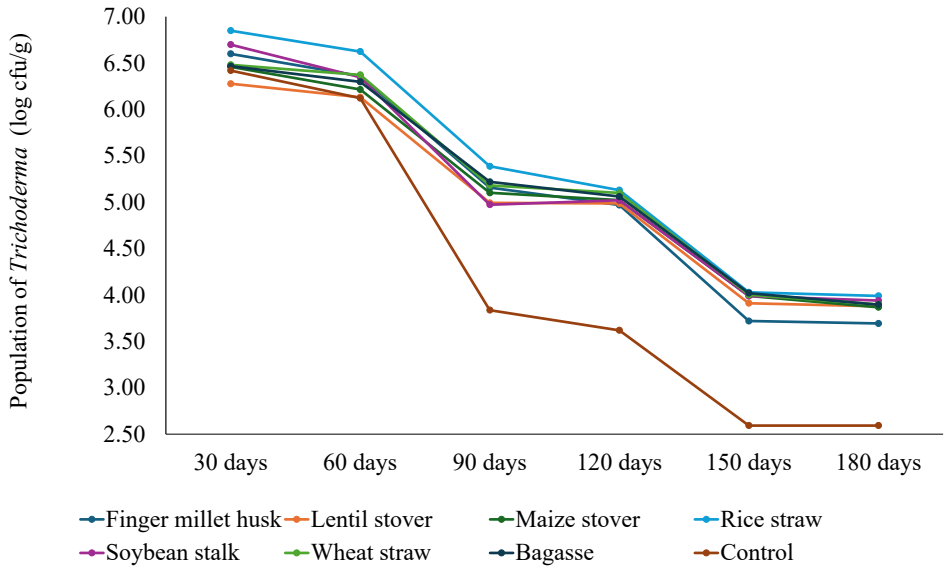
The population of *Trichoderma* in different crop residues remained stable for one month and then decreased very slowly (Figure 2). The number of CFU reduced sharply with time during the six-month period. The pattern of population decline in all crop residue was similar. In control, the population of *Trichoderma* sharply declined and reached 4.33 × 10^3 in 3 months, however, to reduce down the same level of population it took two more months for crop residues. The population of *Trichoderma* reduced slowly from third to forth month in all crop residue and then again reduced sharply for fifth month. On sixth month, population of the fungus declined slowly and remained almost stable.

The population of *Trichoderma* declined continuously in soil in six-months period. Lewis and Papavizas (1984) also observed that population density of *Trichoderma* increased about 10^3 -10^4 fold in soil during the first three weeks of incubation and then

started to decline. Bose and Pan (2009) also reported initial increase in *Trichoderma* population for three weeks (5×10^7 cfu/g soil) and started to decline and reached 7×10^2 cfu/g soil at six weeks. Similarly, Meriles et al. (2006) observed decrease in *Trichoderma* population from 1.85×10^5 to 1.2×10^5 in the following year. *Trichoderma* population was the highest in rice straw followed by soybean stalk and wheat straw.

Figure 2

Population of Trichoderma in soil amended with seven crop residues at different time



Cumagun et al., 2009 reported higher percentage colonization of rice straw by *Trichoderma* in the soil and also observed naturally occurring *Trichoderma* spp. colonizing non-inoculated soil (control). According to Deng et al. (2018) rice straw has high cellulose (39.3%), hemicellulose (24.3 %), lignin (18.8 %) and C/N ratio as compared to soybean stalk. When lignin fibers are damaged, secondary cell walls are exposed, which increase the accessibility of cellulose and hemicellulose, which was advantageous to *Trichoderma* (Deng et al., 2018). *Trichoderma* are reported to be a good cellulose decomposer (Rocha et al., 2016). Although, maize and bagasse contain high cellulose (35-50%) (Daud et al., 2013; Muthuvelayudham & Viruthagiri, 2006) fermentation and textile industries. Trichoderma reesei is an efficient producer of cellulase protein. The comparative study was made on various carbon sources on the production of cellulase using strains of T. reesei QM 9414, 97.177 and Tm3. Pretreatment of sugarcane bagasse and rice straw offers very digestible cellulose and potentially less inhibition. Cellulase production was enhanced by multiple carbon sources because of diauxic pattern

of utilization of substrates. This is the first attempt of combining the synthetic substrate (xylose, lactose the population was low than in rice straw in the study period. However, Meriles et al. (2006) observed highest population of *Trichoderma* in corn residue. The population of *Trichoderma* declined and reached 10^2 CFU/g soil in control plots. Longa et al. (2008)isolated from decayed hazelnut wood in northern Italy in 2000, is a promising fungal agent for biological control of soil-borne plant pathogens. The objective of this research was to characterize the biology and ecology of this fungus, in order to determine its environmental parameter tolerance levels and its behavior in the phylloplane and soil systems. To better characterize T. atroviride SC1, the influences of pH, temperature, water activity and different nitrogen and carbon sources on its in vitro growth were evaluated. T. atroviride SC1 survival was assessed on strawberry leaves under controlled conditions in a greenhouse and in sterilized and non-sterilized soil samples kept at room temperature. Results showed that isolate SC1 is mesophilic and grows best at 25°C. The fungus tolerates a wide range of pH levels, but growth was reduced on alkaline media ($pH \geq 8$ also reported a very low level of native *Trichoderma* spp. about 1 to 3×10^2 CFU/g soil. Similar result was also reported by Lewis & Papavizas (1984).

Trichoderma was applied in the field as conidial preparation in mixture of rice bran and rice husk in 1:5 proportion. Proliferation and establishment of *Trichoderma* in soil depend on inoculum age and amount of inoculum added in relation to the food base (Lewis & Papavizas, 1984). According to Beagle-Ristaino and Papavizas (1985) conidia of *Trichoderma* germinated poorly in soil and sensitive to soil fungistasis and formation of chlamydospores are more important than conidia in survival and proliferation in soil. Similarly, addition of food base support growth and sporulation of *Trichoderma* in soil (Beagle-Ristaino & Papavizas, 1985). In the present study, there was difference in population density of *Trichoderma* sp. based on residue of plant species used. According to (Kerdraon et al., 2019a) microorganisms that proliferate in crop residue depend on the plant (species, genotype, and organ), communities of other organisms that differ in the plant's life and the environment in which it was grown (biotic and abiotic stresses). Even after the plants death, plant species and growing environment affect, microorganisms that colonize the plant residue (Kerdraon et al., 2019b)we used a combined diachronic and synchronic field experiment based on wheat-oilseed rape rotations to test the hypothesis that plant is a structuring factor of microbial communities in crop residues, and that this effect decreases over time with their likely progressive degradation and colonisation by other microorganisms. We characterised an entire fungal and bacterial community associated with 150 wheat and oilseed rape residue samples at a plurennial scale by metabarcoding. The impact of plant species on the residue microbiota decreased over time and our data revealed turnover, with the replacement of oligotrophs, often plant-specific genera (such as pathogens. Diversity and density of microbial communities depends upon

biochemical composition of crop residues which in turn depends on the plant species (Baumann et al., 2009).

Decomposition of crop residue like rice straw and wheat straw produce organic acid in early stage of decomposition. The danger of accumulation of organic acid can be reduced by accelerating decomposition by inoculating micro-organisms like *Trichoderma* (Sharma et al., 2012). *Trichoderma* spp. are lignolytic and cellulolytic saprophytic micro-organisms which can exhibit high N decomposition (12 mg/g straw) along with *Clostridium butyricum* (Lynch & Harper, 1985). Amira et al. (2011) reported that compost inoculated with *T. virens* has higher xylanase and cellulase activities. These activities ultimately accelerate the degradation of cellulose and hemicellulose that help to reduce the time of the decomposition process.

Conclusion

Among the crop residues tested, rice straw consistently supported the highest population of *Trichoderma* throughout the six-month period. The population remained relatively stable during the first month post-inoculation, followed by a gradual decline over time. However, even after six months, rice straw amended plots maintained significantly higher colony-forming units (CFU/g soil) compared to other treatments, indicating its superior suitability as a substrate for prolonged *Trichoderma* survival.

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Conflicts of Interest

There is no conflict of interest.

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