EFFECT OF TRICHODERMA VIRIDE AS BIOFERTILIZER ON GROWTH AND YIELD OF WHEAT

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ABSTRACT

This experiment was conducted to find out the effects of *Trichoderma viride* on growth and yield of wheat at Institute of Agriculture and Animal Science, Lamjung Campus, Sundarbazar, Lamjung during December 2016 – April 2017. The experiment consisted of seven treatments; (T1: Control; T2: Soil + NPK; T3: Soil inoculated *Trichoderma*; T4: *Trichoderma* + FYM; T5: *Trichoderma* + 1/2 NPK; T6: *Trichoderma* + NPK and T7 = *Trichoderma* + NPK + FYM) laid out in completely randomized design (CRD) with three replications. The results showed that *Trichoderma viride* increased the plant height (4.6%), root weight (1.5%), leaf length (0.3%), panicle weight (9.1%), number of grains (3.8%), grain yield (36.5%), biological yield (13.7%), and biomass yield (2.7%) over control; while root length (-17.4%), number of leaves (-8.4%), tiller number (-10.8%), panicle number (-6.7%), panicle length (-8.4%) highlighted the negative impact of *T. viride* on wheat plant. *T. viride* displayed antagonism with inorganic fertilizer. When *T. viride* and NPK were accompanied with farmyard manure, most of the growth and yield parameter showed the highest value. Though *Trichoderma viride* decreases several growth parameters, it still can be used as biofertilizer which increases the grain yield. Using *T. viride* with a full dose of NPK during sowing stage may not be efficient and economical in terms of productivity. Introducing farmyard manure to *T. viride* gives better yield than *T. viride* alone.

KEYWORDS

*Trichoderma viride*, wheat, NPK, grain yield.

1. INTRODUCTION

Wheat (*Triticum aestivum*) is the most extensively grown cereal crop in the world. The optimum temperature for vegetative growth is 16-22 °C and requires about 14-15 °C optimum average temperature at the time of maturity. Temperatures above 25 °C during this period tend to decrease grain weight. Wheat can be grown successfully in those regions where annual rainfall varies from 25 to 150 cm [1]. Wheat is an important non-leguminous crop which requires a high input of chemical fertilizers. The nutrients removal principally NPK by the wheat crop is 227 kg/ha [2]. Nitrogen (N) is the most limiting nutrient for wheat production that affects the speedy plant growth and improves grain yield [3].

*Trichoderma* species are the fungi that are present in nearly in all soils and other habitats. *Trichoderma* species include *T. harzianum*, *T. viride*, *T. koningii*, *T. hamatum* and other species [4, 5]. *Trichoderma* colonizes the root surface or cortex and proliferate better when there are abundant healthy roots [6]. They have evolved numerous mechanism for both attacks of the fungi and for enhancing the root growth [7]. *Trichoderma* has the capacity to produce antibiotics, parasitize other fungi, and compete with deleterious microorganisms which were considered to be the basis for how *Trichoderma* exert beneficial effects on plant growth and development [8]. The benefits of *Trichoderma* species in improving plant growth can be realized through several mechanisms which include mycoparasitism, antibiotics, degradation of toxins, inactivation of pathogenic enzymes pathways, resistance against pathogens, enhanced nutrient uptake, solubilization, sequestration of inorganic nutrients and enhanced root hair development [9, 10]. *Trichoderma* helps to increase plant hormone which helps to increase root growth and root hair formation that results in the more efficient use of nitrogen, phosphorus, potassium and micronutrients and increase seedling vigor and germination [11].

A group researcher documented that *Trichoderma harzianum* and *Trichoderma asperellum* are highly rhizosphere competent and able to stimulate the growth and immune defense of plants [8]. Some of researcher reported that *Trichoderma harzianum* has the ability to solubilize phosphate and micronutrients that could be made available to plant [12]. *Trichoderma harzianum* and *Trichoderma viride* enhanced rice and tomato root and shoot length [13, 14]. Seed germination, root length, shoot length, fresh weight, dry weight, and vigor index were significantly increased by *T. viride* and *P. fluorescens* [15]. Research has found that the corn plant colonized with *Trichoderma* strain T22 requires 40% less nitrogen fertilizer than the plants which lack these fungi and, hence, helps to minimize the damage to the environment [16]. Some group researchers also documented that recommended dose of NPK and 50% biofertilizer and compost + 50% NPK showed similar effects on growth, dry matter and yield of mustard [17]. The seed yield per plant was increased by 5.34% over the recommended dose of NPK applied. *Trichoderma longibrachiatum* has a higher potential of parasitic and lethal effects against *Heterodera avenae*, but its effects on wheat are fairly high in promoting plant growth and nematode control [18]. One strain of *Trichoderma* increases the numbers of deep roots at as much as a meter below the soil surface [19]. These deep roots cause crops like corn and ornamental plants like turf grass to become more resistant to drought.

The application of *Trichoderma harzianum* T22 increased all measured parameters such as growth parameters, chlorophyll content, starch content, nucleic acids content, total protein and phytoregulation of maize plant [20]. Another group researchers found that *Trichoderma* was able to enhance rice growth components such as plant height, leaf number, tiller number, root length and shoot fresh weight [21]. Other researchers reported that elicitors released by *Trichoderma* are involved in triggering expressions of defense protein within the plant to induce plant immunity against pathogens and, in turn, improve plant growth [22]. *Trichoderma koningii* that colonized the roots of *Lotus japonicas* was found to produce is flavonoid and phytalexin and stimulate plant dry weight [23]. Since a few works is done to understand the impact of *Trichoderma* and wheat, this study on the wheat plant is done to understand its role as fungicides and/or growth promoters.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experimental study was carried out in the premise of Institute of...
Agriculture and Animal Science, Lamjung Campus, Sundarbanzor in the western mid hills of Nepal during December 2016 – April 2017 [24-28]. This place has a humid tropical climate with an annual rainfall of 280 cm. The geographical position of the farm is at the latitude of 29° 8' 41” N and longitude of 89° 2' 43” E and elevation of 610 masl.

The soil sample was sent to Soil Laboratory of Soil Management Directorate, a Nepal Government establishment, Kathmandu, Nepal. The pH, nitrogen, organic matter, phosphorus, and potash content of the soil sample were evaluated. All the chemical needed for the analysis in soil lab were procured from BDH Company (UK or India) and E. Merck (Germany or India). For pH, 20 g of soil was mixed with 20 ml of distilled water in 1:1 ratio. After mixing for 1 minute, the solution was allowed to stand for 1 hour. pH meter was dipped in the stirred soil solution and the pH was measured [29,30]. 1 g of soil (particle size 0.2 mm) was scooped into a 500 ml Erlenmeyer flask using standard scooping techniques. 10 mL of IN Na2Cr2O7 solution and 20 mL of concentrated sulfuric acid was added and were allowed to react for 30 minutes. 200 ml distilled water, 30 drops diphenylamine indicator and 0.2 g NaF were added to the flask. 0.5 N ferrous ammonium sulphate solution was used to titrate the blank and the sample. Finally, the organic matter was calculated [31-33].

Kjeldahl method was used for the determination of total nitrogen. Available phosphorous was determined by Bray and Kurtz No. 1 Method. For the determination of potassium, Flame Photometer Method was used.

2.2.2 Trichoderma soil inoculation

10^6 cfu/ml conidial suspension of Trichoderma viridae was diluted in 5 liters of water so as to prepare a solution strength of 2X10^4 cfu/ml. For each pot, 100 ml of solution was used which accounted 2X10^4 cfu/ml. Per pot, 100 ml of the solution was used to drench the soil per pot [11, 24].

2.2.3 Sowing, Irrigation, Weed control and Harvesting

Sowing and light irrigation were done on December 26, 2016. After the complete germination, the wheat plants were thinned out leaving only four wheat plants in each pot. Plant to plant distance of 6 cm was maintained. Irrigation with 250 ml of water was done on an interval of two days which subsequently decreased to once a week when plants neared to harvest.

Hand weeding was done on 35th and 55th days of sowing. Aphid infestation was controlled by spraying detergent water (2 teaspoon detergent per liter of water) to wheat plants for two weeks on alternate days. When the aphid infestation was not controlled, Rogit (Dimethoate 30% EC; i.e. Emulifiable concentrate; HPM Chemicals & Fertilizers Ltd, Delhi, India) were applied. Harvesting was done manually on April 17, 2017 (113 days after sowing (DAS)).

2.3 Data Collection and analysis

Plant height (cm), leaf number, leaf length and width (cm), number of tillers per plant, panicle number, panicle length (cm) and weight (g), number of grains per plant, root length (cm), dry root weight (g), dry shoot weight (g), total biomass (ton/ha), yield per plant, grain yield (ton/ha) and biological yield (ton/ha) were taken. MS-Excel worksheet version 15 was used to record the data and the simple statistical analysis as well as table, charts, and graph. Further statistical analysis to determine the significance (at a level of 5%) among various treatments was performed using Genstat version 15.

3. RESULTS

The soil pH was found to be slightly acidic 6.0, organic matter 2.81% (medium), nitrogen 0.14% (medium), phosphorus 216.68 kg/ha (high) and potash 53.49 kg/ha (high). Taking T1 (only soil) as control, plant height showed the highest increase of 14.5% in T2 (only inorganic fertilizer), but when mixed with Trichoderma as in T6, the height increase was only 4.6% which was also seen in T3 (only inorganic fertilizer), T4 (Trichoderma + FYM) and T5 (2X10^4 cfu/ml). Interestingly an increase of 11.2% was observed when half of NPK was added and were allowed to react for 30 minutes. 200 ml distilled water, 30 drops diphenylamine indicator and 0.2 g NaF were added to the flask. 0.5 N ferrous ammonium sulphate solution was used to titrate the blank and the sample. Finally, the organic matter was calculated [31-33].

Kjeldahl method was used for the determination of total nitrogen. Available phosphorous was determined by Bray and Kurtz No. 1 Method. For the determination of potassium, Flame Photometer Method was used.

The treatments were control (T1), only inorganic fertilizer 120:80:80 NPK kg ha-1 (T2), Trichoderma soil inoculated (T3), Trichoderma + 10 tha-1 FYM (T4), Trichoderma + 60:40:40 NPK kg ha-1 (T5), Trichoderma + 120:80:80 NPK kg ha-1 (T6), Trichoderma + 120:80:80 NPK kg ha-1 + 10 tha-1 FYM (T7). For control, no manures and fertilizers were applied. Fertilizer sources were Trichoderma viridae, FYM and chemical fertilizer NPK (Urea (CO(NH)2)2 (HiMedia Laboratories, Mumbai, India) for nitrogen (N)), Muriate of Potash (KCl) (HiMedia Laboratories, Mumbai, India) for potassium (K) and Diammonium phosphate ((NH4)2HPO4) (HiMedia Laboratories, Mumbai, India) for phosphorous (P). Trichoderma viridae was used as biofertilizer which was obtained from AASRA Research and Education Academy Counsel, Biratnagar, Nepal.

FYM was used from the IAAS Campus Farm at the rate of 10 ton/ha. Farmyard manure was tested in lab for the presence of Trichoderma and it was found to contain nearly 10^6 cfu/ml of conidia. As far as species is concerned most of them was T. viridae while a few were T. harzianum. For enumerating the viable spores of Trichoderma in a formulation, the serial dilution was done with Tween 20 and the dilution was restricted to 10^-6.

The tips was changed for each dilution without fail. The higher dilution of 10^-6 was spread on Potato Dextrose Agar Plate (HiMedia Laboratories, Mumbai, India) in triplicate. Plates with colony count of 8-80 only was considered for enumeration [35].

To estimate the amount of fertilizer and FYM for a single plant, plant population per hectare (Pp) was calculated using the formula. The total amount of fertilizer and FYM required for one hectare was divided by plant population per hectare. Thus, the needed amount of fertilizer per plant was obtained.

\[
Pp = \frac{10,000 \times m^2 \times \text{number of seeds per stand}}{\text{Product of spacing (m²)}} \quad \text{(Eq. 1)}
\]

The product of spacing used was 18 cm X 6 cm while the number of seed per stand was 1. This resulted in plant population of 925926 per hectare.

Fertilizer per plant = \frac{\text{Amount of fertilizer per hectare}}{\text{Plant population per hectare}} \quad \text{(Eq. 2)}

Table 1: Effect of treatments on growth performance of wheat in terms of measured values and standard errors

| Treatment       | Plant height (cm) | Root length (cm) | Dry root wt (g) | Dry Shoot wt (g) | Biomass (ton/ha) |
|-----------------|------------------|------------------|-----------------|------------------|-----------------|
| T1              | 65.97±2.53       | 19.98±1.64       | 2.59±0.06       | 0.09±0.09        | 2.99±0.13       |
| T2              | 73.55±3.39       | 21.98±2.35       | 3.01±0.10       | 0.14±0.07        | 4.47±0.07       |
| T3              | 69.03±2.01       | 14.25±1.81       | 2.73±0.25       | 0.21±0.12        | 2.13±0.36       |
| T4              | 71.26±2.40       | 14.91±1.11       | 3.01±0.10       | 0.23±0.12        | 2.79±0.24       |
| T5              | 73.35±3.74       | 11.35±0.56       | 1.94±0.04       | 0.34±0.18        | 3.99±0.29       |
| T6              | 69.03±0.76       | 12.67±0.51       | 0.89±0.05       | 3.30±0.25        | 3.88±0.27       |
| T7              | 67.85±0.15       | 16.73±0.12       | 0.31±0.12       | 4.39±0.18        | 4.48±0.27       |

CV% 5.8 11.8 21.5 15.4 14.6

F value 0.100 0.006 0.008 <0.001 <0.001

T1: Control; T2: Soil + NK; T3: Soil inoculated Trichoderma; T4: Trichoderma + FYM; T5: Trichoderma + 1/2 NK; T6: Trichoderma + NPK and T7 = Trichoderma + NPK + FYM

Trichoderma profoundly exhibited the root length inhibitory (17.37%) nature as shown by T3 compared to control (T1) which was in line with T4 (Table 1). The efficacy of NPK was reduced (T5 and T6) by Trichoderma. Root length was lower than control in T4 (2.26%) and T5.
Inorganic fertilizer increased root length by 11.16% (T2) and 14.96% (T7) over control (T1). The antagonistic relationship of Trichoderma and chemical fertilizer was also observed in root length. Trichoderma viride exhibited increase (1.49%) in root weight as shown by T3, while the addition of farmyard manure with Trichoderma (T4) slightly increased (5.55%) the root weight. Inorganic fertilizer increases root weight by 77.2% (T2) over control (T1). The root weight increased by 54.26% with Trichoderma combined to lower NPK (T5) in contrast to T3 showing an increase of 20.3%. The highest increase of 82.68% was observed in T2. Despite the lower root length than control in T3, T4, and T5; root weight was higher which was due to the higher root density T3, T4, and T5.

Least number of the leaf (Table 2) was seen in Trichoderma treatment (T3) which was found 8.4% less than control. Such type of result was also observed in T6 (NPK and Trichoderma) with a 7.7% decrease in leaf number over control. A rise of 40.1% in leaf number was seen in T7 (Trichoderma + Full NPK + FYM) which showed a greater improvement over the 5.5% increase observed with Trichoderma and FYM. The highest number of leaves was found in T7. Trichoderma (T3) didn’t show any observable change in leaf length and width over control (T1) while NPK as T2 and T7 showed a high increase of 11.5% and 14.8% in leaf length respectively and 30% and 43% in leaf width respectively. The antagonism of NPK and Trichoderma was observed even in leaf length (Table 2).

| Treatment       | Leaf number | Leaf length (cm) | Leaf width (cm) | Tiller number | Panicle number |
|-----------------|-------------|------------------|-----------------|---------------|---------------|
| T1              | 12.55±0.57  | 26.93±0.55       | 1.06±0.01       | 3.03±0.08     | 1.25±0.14     |
| T2              | 13.66±1.54  | 30.03±0.41       | 1.31±0.03       | 3.16±0.08     | 1.66±0.22     |
| T3              | 11.9±1.56   | 27.0±0.3         | 1.0±0.00        | 2.79±0.29     | 1.167±0.08    |
| T4              | 13.2±1.0    | 28.6±1.37        | 1.07±0.07       | 3.41±0.17     | 1.33±0.01     |
| T5              | 13.33±0.58  | 28.67±0.44       | 1.07±0.03       | 3.54±0.22     | 1.33±0.08     |
| T6              | 11.58±0.17  | 28.2±0.02        | 1.07±0.04       | 3.08±0.08     | 1.33±0.22     |
| T7              | 17.58±0.22  | 30.93±1.16       | 1.43±0.07       | 4.00±0.36     | 1.5±0.14      |
| CV%             | 8.1         | 12.4             | 15.8            | 8.8           | 10.7          |
| F value         | 0.009       | 0.036            | 0.001           | 0.013         | 0.361         |

Table 2: Effect of treatments on growth performance of wheat in terms of measured values and standard errors

Grain yield per hectare was 1.53 ton/ha in T7 (Trichoderma + FYM + NPK) which showed a 75.8% increase over control (T1). This increase was slightly higher than T2 (69.3%) which valued to 1.47 ton/ha. Considering half of the NPK used in T5, the yield of 1.44 ton/ha with an increase of 65.9% was a good output. Trichoderma treatment (T3) could only increase 15% of grain yield while Trichoderma + farmyard manure (T4) accounted an increase of 41.8% (Table 3). A full dose of NPK and Trichoderma mixture (T6) showed a poor yield of 1.33 ton/ha with an increase of 52.4% which was much lesser than T5 where half of the NPK was used with Trichoderma.

Total biomass was highly affected in T3 and T4 and illustrated the slight increase in biomass with Trichoderma either used alone or in combination with farmyard manure (Table 1). The highest increase of 73.0% was observed in T7 which was slightly over T2 (72.6%). Introducing Trichoderma with half of NPK (T5) gave a higher biomass (54.1%) than T6 (49.8%). As far as biological yield was considered, Trichoderma in T3 and T4 showed only 13.7% and 18.7% increase over control respectively, while T2 (only chemical fertilizer) and T7 (NPK + FYM + Trichoderma) showed the higher increase of 70.5% and 71.2% respectively. The antagonistic relationship of Trichoderma and chemical fertilizer was clearly visible in T5 and T6. The increase in dry biomass with Trichoderma treatment was supported by Cuevas [25] in tomato.

4. DISCUSSION

The impact of Trichoderma on plant height was in harmony with the findings of a group researcher where T. viride inoculated coton plants increased shoot length when compared with the control [15]. The stunting of T6 (Trichoderma+ NPK) and T7 (Trichoderma+ Full NPK + FYM) may be due to enhanced ammonium uptake, resulting in ammonia toxicity. A good increase in height of T5 having half NPK and Trichoderma as a treatment approves the logic of ammonium toxicity [26]. In case of plant height, it is evident that there is an antagonistic relationship between chemical fertilizer and Trichoderma possibly because of ammonium toxicity. As a result, plant height is adversely affected. Less is the chemical fertilizer, lesser adversely observed.

The negative impact of Trichoderma on root length is also state in a study of Arabidopsis [27]. The antagonistic feature of chemical fertilizer and Trichoderma is supported by the findings of Badar and Qureshi on Vignamungo [28]. Trichoderma showed increased root and shoot growth in this pot experiment. The stronger root system leads to an improved uptake of water, minerals, and nutrients when the root surface area responds to nutrient limitation circumstances [16].

The negative impact of Trichoderma on the number of leaves is also reported some researcher on maize leaves [20], it illustrates the negative impact of Trichoderma on leaf number of the wheat plant. The number of leaves displays an antagonistic relationship between inorganic fertilizer and Trichoderma. Farmyard manure facilitates NPK and Trichoderma mixture which surprisingly increases leaves number.

The inhibitory nature of Trichoderma for tiller number, panicle number, and panicle length is supported by the observation of rice while contradicted by the studies of another studies in rice [25, 29,30]. Trichoderma shows an increase in panicle weight as well as the number of grains. The findings of Trichoderma over control is aligned with one more study [31]. High chemical fertilizer with Trichoderma is inhibitory for panicle weight and the number of grains.
The result of grain yield in *Trichoderma* is supported by the study of a group researcher which shows a significant increase in the yield of wheat of about 29% in Jaipur and 36% in Kota [31]. Trichoderma hazarium has the ability to solubilize phosphate and micronutrients that could be made available to plant [12]. Though the total combination of *Trichoderma*, farmyard manure, and full NPK yield was 75.8%, the yield increases of 65.9% with half of NPK and *Trichoderma* could be basic highlight considering that only NPK treatment yield was 69.3% higher. In this experiment, the increase in yield can also be attributed to the application of *Trichoderma* bioformulation along with FYM which helped increasing the colonies by providing nutrient to *Trichoderma* thereby increasing the plant growth and yield of wheat [31].

Though our finding of *Trichoderma* is as a growth promoter with certain limitations, *Trichoderma* has been fully supported as a growth promoter on numerous cultivated plants [16, 32, 33, 36]. This specified the potential use of the biofertilizers as a reasonable alternative for crop production, with a minimization of the ecological impact and improvement of soil ecology.

5. CONCLUSION

*Trichoderma* shows a slight increase in the plant height, panicle weight, number of grains, grain yield, biological yield, and biomass yield over control; while root length, number of leaves, tiller number, panicle number, panicle length highlight the negative impact of *Trichoderma* on the wheat plant. *Trichoderma* shows antagonism with inorganic fertilizer. In most of the parameters, more is the inorganic fertilizer with *Trichoderma* show higher antagonism. When *Trichoderma* and NPK are accompanied with farmyard manure, most of the growth and yield parameter shows the highest value, but the yield was slightly higher than NPK alone treatment. This finding indicates that while sowing seed, the use of *Trichoderma* with FYM and NPK may not improve the yield over NPK to a greater extent. Hence it is indicated that *Trichoderma* viride can be a growth promoter and be used as a biofertilizer.

CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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