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Isolation of Trichoderma Harzianum from Soil and its Effect on Germination Potential of Selected Cereal Crops

*Mustapha Isah, Abdullahi M. Labbo, Nura M. Bello, and Sumayya A. Ayuba

Department of Biochemistry, Sokoto State University, Sokoto -Nigeria

***Corresponding author's email:**

mustyeeser029@gmail.com/

mustapha.isah@ssuedu.ng

The fungus Trichoderma is known due to its versatility for promoting crop growth, improving the nutrient absorption and increasing the grains yield. The work was aimed to isolate the fungus Trichoderma and study its effect on the germination of selected cereal crops. The fungus was isolated from soil samples around Sokoto State University, Sokoto (SSU) using Potato Dextrose Agar (PDA) media and microscopically identified as *Trichoderma harzianum*. The effect of *Trichoderma harzianum* on germination was observed against Sorghum, Maize, Wheat, Barley and Millet respectively. The seeds were subjected to germination with 98% relative humidity and 12 hours' light exposure. The percentage germination (PG) using 1% fungal concentration was found to be 58.00% ± 3.00, Sorghum 37.33% ± 1.33, Wheat 36.00% ± 4.00, Barley 28.33% ± 5.87 and Maize 17.33% ± 2.40 while PG for 2% fungal concentration were 58.93% $\pm$ 2.96 for Millet, Sorghum was 38.93% $\pm$ 2.96, while Wheat 37.00% $\pm$ 5.13 and Barley was 25.00% $\pm$ 1.73. The seeds treated with *T. harzianum* were considered effective since it provided grains germination percentage superior compared to the control.

Keywords: *Trichoderma harzianum*, Germination potential, Cereals, Bio fertilizer, Soil fertility

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1. Introduction

The genus *Trichoderma* is a diverse group of free-living fungi in the family *Hypocreaceae*, commonly present in all soils. In soil, they are frequently the most prevalent culturable fungi [11]. They are favored by the presence of high levels of plant roots, which they colonize readily [13]. Some strains are highly rhizosphere inhabitant able to colonize and grow on roots as they develop. In addition to colonizing roots, *Trichoderma spp*. attack, parasitize and gaining nutrients from other fungi. Since *Trichoderma spp*. grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant growth [26]. These ascomycetes fungi are opportunistic, avirulent plant symbionts inhabiting root ecosystems and parasiting other groups of fungi. *T. harzianum* and *T. hamatum* reproduce by chlamydospores and ascospores. They proliferate better at (25–35°C) with pH range of 5.5–9.0 [6] [36] [39]. It colonizes several ecological niches where they play pivotal roles. They have been earlier recognized as effective biocontrol agents of plant-pathogenic fungi, producers of secondary metabolites of medical importance and agents of bioremediation. Similarly, their ability to degrade lignocellulosic biomass to produce second-generation biofuels and other value-added products have been reported [reported [10].

Many studies have been published reporting the ability of Trichoderma species proliferating plant growth, parasitizing other fungi, producing antibiotics, and competing with harmful plant microorganisms as well as acting as biocontrol agents and bio fertilisers [1] [5] [14]. Other studies testified the application of *Trichoderma* species served as bio stimulants and stimulate plant defence mechanisms [8] [35] [37]. Harman [19], described the traits that were considered as the basis of how these species induce beneficial factors for plant growth. Conversely, it is obvious that other strains possess significant influence pertaining plant growth as well as crop production.

T. harzianum might serve as an alternative in suppressing wilt pathogens thereby increasing the plants' yields [31]. Another report of increasing Chlorophyll content upon coating the seed with *T. harzianum* was reported [33]. Likewise, other Trichoderma species have the capability of promoting plant growth due to different mechanisms, which include phosphate solubilization, induction of micronutrient and other minerals which play a vital role in plant development and improving the nutrient uptake [22], plant defense capability against both abiotic or biotic type of stress among others [27]. The fungal *Trichoderma* have been found to be strong opportunistic invaders, fast growing, prolific producers of spores and also powerful antibiotic producers even under highly competitive space, nutrients, light and also involved in formation of ROS [20] [30] [34].

In this study, the fungus was isolated in three different soil sites of which only one colony related to Trichoderma was microscopically identified as *Trichoderma harzianum* and assessed its effect on germination potential on selected cereal crops.

2. Materials and Method

2.1 Sample Collection

Soil samples were collected from three different sites of Sokoto State University (SSU) that include: 60 offices complex, female student hostel and Onion plantation site were each sample was designated as X, Y and Z respectively. Ten grams (10g) of the soil sample collected was suspended into 20ml of sterile distilled water and then allowed to stay for 30 minutes according to [24]. The cereal samples were purchased from Sokoto central market and washed with 2% sodium hypochlorite then rinsed with sterile distilled water and dried in a glass cabinet.

2.2 Media Preparation

Potato dextrose agar (PDA) media was used for cultivation of fungi in the present study. Thirty-nine gram of PDA powdered was weighed and dissolved into 1 liter of distilled water and stirred using a magnetic stirrer in a mild temperature until the mixture dissolved. The mixture was then autoclaved at 121°C and 15 lb pressure for 30 minutes [24]. The prepared agar was allowed to cool around 50°C. A biocidal; 0.25g of chloramphenicol was then added in to the media as modified [4]. 15-20ml of the prepared agar media was added to each petri dish and allowed to solidify, then kept for future use.

2.3 Sample Preparation

Ten grams (10g) of the soil samples suspended into 20ml of sterilized distilled water and stirred; 0.1ml of the supernatant was used for serial dilution up to 10^{-3} was made according to [4].

2.4 Cultivation of *Trichoderma Spp*.

Half mililitre (0.5ml) of each serial dilution was collected according to [25] and spread on the agar surface using an L shape glass rod. The plates were incubated in inverted position at 25 °C for three (3) days.

2.5 Isolation of *Trichoderma Spp*.

After three (3) days of incubation, different colonies were observed. To form a pure colony, a new petri dish containing basal PDA media was used for the isolation. A sterile sharped, straight inoculating wire was used and gently scratched on the grown colony formed from the former petri dish and transferred to the new PDA media petri dish in zigzag drag pattern and then incubated in inverted position at 25 °C for seven days.

2.6 Microscopic identification Using Colony Morphology

The colony formed after seven days were clearly labelled and different isolates were accessed on the preliminary identification via the morphology; as the isolate was viewed microscopically using the ×40 followed by ×100 objective lens and then snapped with camera and compared with atlas of microbiology.

2.7 Collection of Spores

After seven days of incubation, *Trichoderma* spp. spores were collected by topical scratch and addition of sterile distilled water and filtered through sterilize gauze. The obtained concentration was adjusted to 10^6 spores/ml according to [9].

2.8 Seed Germination Assessment

The selected cereal crops (maize, sorghum, millet, barley and wheat) which were disinfected with sodium hypochlorite (1%) for two (2) minutes, then, rinses with sterile distilled water. Each seeds were treated with spores collected from the fungal isolates and 100 seeds were counted and plated on blotter paper, using 9cm petri dishes with two layers of moist filter papers then, enclosed with a third moist filter paper. Untreated seeds were used as control [7] [16]. The seeds were kept in a growth chamber with 98% humidity followed by 12-hour exposure to light for 72 hours and then germinated seeds were counted.

2.9 Statistical Analysis

All the data collected were subjected to analysis of variance test (one-way ANOVA) using In Stat statistical software.

3. Results and Discussion

3.1 *Trichoderma Species* Isolated from Soil Samples.

The *Trichoderma specie* isolated from the soil sample was identified as *Trichoderma harzianum*. Other fungi isolated was *Aspergillus niger* as their micro/macro characteristics appearance on PDA, and the *Trichoderma harzianum* appearance was compared with its appearance on the atlas of microbiology. Photo micrograph and the name of the organism is shown in Table 1.

Table 1: Morphological Identification of Fungal Isolate from Soil Samples On PDA

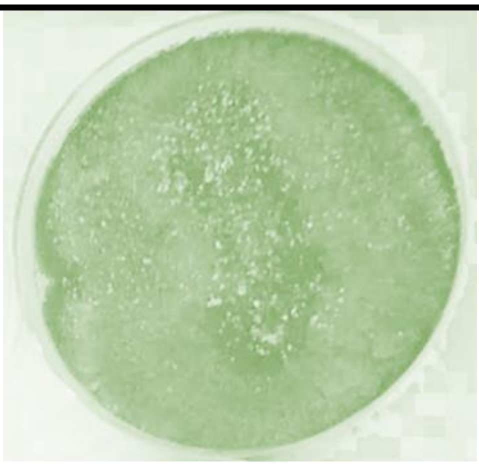
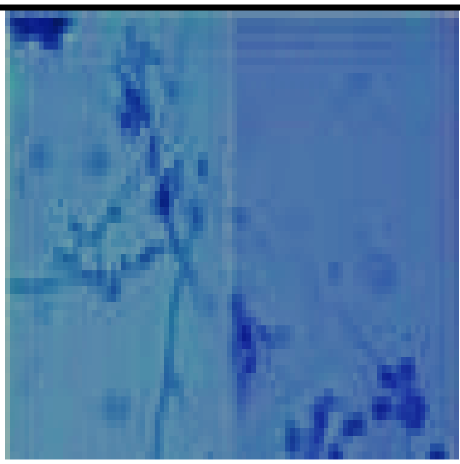
Appearance on PDA	Photo micrograph	Probable organism
		*T. harzianum* (H1)
Morphology		
Colonies are fast growing, at first white and velvety. Later developing yellowish-green to deep green compact tuffs, often in small areas branched, irregular vermiculate, bearing clusters of divergent and irregular bent, rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of phialides. Conidia are mostly green. The conidiospores branches are less compact when viewed microscopically.		

Table 2: Germination Percentage of Selected Cereal Crops Treated with *T. Harzianum*.

Cereal crops	Control %	*T. harzianum* 1 %	*T. harzianum* 2 %
Maize	2.67 ±2.40*	17.33±2.40**	15.00±1.15**
Wheat	17.33±2.91*	36.0±4.00**	37.00±5.13**
Sorghum	19.33±5.81*	37.33±1.33**	38.93±2.96**
Millet	21.67±4.17*	58.0±3.00**	58.0±12.16**
Barley	7.67±4.25*	28.33±5.87**	25.0±1.73**

Values are expressed in mean ± SEM of three replicates. Values in columns having different superscript differ significantly at p <0.05 (one-way ANOVA). ** = more significant * = significant.

3.2 Maize, Wheat, Sorghum, Millet and Barley Seedling Growth by the Treatment of Seeds with *Trichoderma Harzianum*.

In the percentage germination potential, *T. harzianum* was superior to the other seeds used as control. Maize had a PG of 17.33% and 15.0%, wheat a PG of 36.0% and 37.0%, sorghum a PG of 37.33% and 38.93%, millet a PG of 58.0% and barley a PG of 28.33% and 25.0%. Analysis of variance showed significant differences with p<0.05 between the seedlings treated with *T. harzianum* and the control as shown in table 1.

In this study, *Trichoderma harzianum* was isolated from soil samples obtained from SSU surroundings. For the isolation of *T. harzianum*, the colonies were fast growing, developed a whitish color within 1-2 days, which turned globose dark at the center then dull green with compact and dully conidiophores throughout the petri plates. Spherical, smooth and hyalia conidia were formed on the conidiophores when viewed microscopically. Similar results were observed by Harman *et al.,* [18] who reported that *Trichoderma species* are ubiquitous saprobes, common in soil and root ecosystems. Also Zeilinger *et al.,* [40] reported that *Trichoderma species* are easily isolated from soil, decaying wood and other organic materials.

In this study, the percentage germination of five (5) cereal crops were determined using 1% and 2% fungal concentrations. The result revealed that there are no statistical differences between the germination percentage of these two concentrations in all the cereals used in this analysis, but highly significant differences were recorded in comparison with the control in each and every sample used. The reason for the enhanced germination is predicted physiologically and biochemically by stating that water absorption and presence of hormones are fundamental for the physiological processes for germination to occur [41]. For this reason, facilitating this germination in the presence of Trichoderma might be due to the increase in growth hormones, water absorption and activation of metabolic activities induced by this amazing fungus. As it was reported by Hoyos-carvajal *et al.,* [22] that, *Trichoderma spp.* can promote beneficial effects of plants' germination and seedling emergence, due to the solubilization of nutrients available for root uptake. Other authors had testified some strains of *Trichoderma spp*. increase the percentage of germination of cereals, such as maize and chicken pea [3] [23] [38]. Correspondingly, when tomatoe seeds were bioprimed with *T. harzianum* and *T. asperellum* an increase in seedling height, weight of fresh and dry shoots and roots were significantly recorded [28]. It was also reported the application of *Trichoderma species* with growth promoting capabilities in plant helps to reduce chemical pesticides and fertilizers input in agriculture [26]. The author further suggested that; the growth stimulating capability of the *Trichoderma* is associated with the induction of plant's growth hormone known as auxin.

In the specific case of this work, maize, wheat, sorghum, millet and barley increase in percentage germination is similar to the suggestion made that mention the probable mechanism to promote growth might be direct as a result of production of hormones that stimulate plant growth. In the studies conducted by Gravel *et al.,* [15], *Trichoderma spp*. was confirmed as an indole-acetic acid (IAA) inducer, promoting the growth of *Solanum lycopersicum*. In similar research, treatment of maize seeds with *T. harzianum* solution before sowing showed a significant increase in GA3 and IAA contents whereas decreasing the ABA content Akladious and Abbas, [2].

Therefore, this study, revealed the maize germination percentage was effectively increased from 2.67% in control to 17.33% in *T. harzianum* and 15.0% in *T. harzianum* using 1% and 2% of spore concentrations which show a highly significant result in both concentrations compared to control. For wheat germination, percentage was significantly increased from 17.33% in control to 36.0% in *T. harzianum* 1% and 37.0% in *T. harzianum* 2%. For sorghum, germination percentage was effectively increased from 19.33% in control to 37.33% in *T. harzianum* 1% and *T. harzianum* 2%. For millet germination, percentage was efficiently increased from 21.67% in control to 58.0% in *T. harzianum* 1% and 58.0% in *T. harzianum* 2%. Similarly, the barley germination, percentage was increased from 7.67% in control to 28.33% in *T. harzianum* 1% and 25.0% in *T. harzianum* 2%. But no significant differences were recorded as a result of spore concentration of 1% and 2%.

This showed that there was an increase in PG on seeds treated with *T. harzianum* when compared to control. The present work is similar with the findings of Petrisor *et al.,* [32], who described *in vitro* effect of *Trichoderma* species and salicylic acid against germination rate on selected vegetables. This work is said to be reliable considering the studies conducted by Hajieghrari [17], Hosomi *et al.,* [21], Mokhtar & Dehimat, [29], and Shoresh *et al.*, [38], describing how *T. harzianum* had been tried for increasing the percentage germination of wheat and maize. Besides the increase in germination potential, Akladious and Abbas [2] reported that; upon comparing the protein content of the *T. harzianum* treated maize with that of control, the result showed newly formed protein bands appeared using electrophoretic analysis.

4. Conclusion

Trichoderma harzianum had provided significant increase in percentage germination and prospectively this could increase the plant growth because germination is the basis of plant growth and development and definitely might increase the agricultural output as a result of inoculating the seeds with this bio agent as observed in this finding. The future research could also be conducted and focusing on its effect on nutritional compositions upon application of such important fungal species to cereals.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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