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Indigenous *Trichoderma* Isolated from Maize Rhizosphere with Potential for Enhancing Seedling Growth

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Abstract: ***Plants and microorganisms interaction often occur in the rhizosphere and improve plant growth. In this research, we tried to isolate Trichoderma from maize rhizosphere and evaluated for its role in stimulating of seedling growth. Three isolates of this fungus were collected from maize plantation in Maros, Takalar, and Jeneponto regencies, Sulawesi, Indonesia. The tree isolates have same characteristic apparent with thick and velvety in texture, dark green in color, and rapid growth in PDA medium. Comparing with control, the application of these isolates offered no any impact on seed germination, but significantly increased root length, shoot length, dry root weight, and dry shoot weight. No significant different between isolates, except the root treated by Jeneponto isolate was more longer than that treated by Maros and Takalar isolates. These data showed the potential of this indigenous Trichoderma as agent of plant growth.***

Keywords: Indigenous *Trichoderma*, Maize, Rhizosphere, Seedling

1. Introduction

Trichoderma species are cosmopolitan free-living fungi, found in all types of soil and decayed plant tissues (Rahman et al., 2011; Schuster and Schmoll, 2010; Mariola et. al., 2007) and can colonize the roots of many plants as opportunistic avirulent plant symbionts (Harman et al., 2004). With this mechanisms of root and *Trichoderma* relationship permit the plant to adapt to abiotic stresses, nutrient uptake, solute transport as well as for disease infestation (Hermosa et al., 2012; Rosmana et al., 2015). Their processes are facilitated by the induction of cell wall extention and expansion, secondary root development, higher photosynthetic rate and activation of defense system (Contreras-Cornejo et al., 2009; Samolski et al., 2012; Shoresh et al., 2010). In addition, the interaction between root and *Trichoderma* play role in phytohormone signaling (Hermosa et al., 2012).

The capacity of *Trichoderma* to enhance maize growth have been much studied. This *Trichoderma* can promote seedling emergence, final stand and dry weight of maize (Stewart and Hill, 2014). Number of leaves per plant, height of plant, length of root, area of leaves, fresh and dry weights of shoots and roots were also increased, when *Trichoderma harzianum* applied to the soil (Akladious and Abbas, 2012). Even, the research of *Trichoderma* on maize have been documented, looking for specific strain adapting to local condition is still needed. Each *Trichoderma* would have a specific type of influence on plant physiological and biochemical processes depending on the strains (Nawrocka and Małolepsza, 2013).

The use of *Trichoderma* on maize grown in Sulawesi where its climatic condition is more drier compared with other parts of Indonesia would offer an important economic implication such as shortening the maize growth period as well improving maize vigor to overcome biotic and/or abiotic stresses. For this reason, we planed to isolate this *Trichoderma* from maize grown in Sulawesi and to evaluate for its ability for enhancing maize seed germination and seedling growth.

The primary objective of the research was to characterize the *Trichoderma* isolated from maize rhizosphere grown in dry region and analyze its impact on germination rate, root elongation, plant height and dry weight of the seedlings.

2. Materials and Methods

Isolation of *Trichoderma*.
Soil sample of about 200 g were respectively taken from maize rhizosphere at five different places of maize field in Maros, Takalar and Jeneponto regencies, Sulawesi, Indonesia. This sample from each regency was then mixed and by using of dilution method, Trichoderma was isolated from mixing soil. One gram of soil, was taken and crushed before placed in a test tube containing 9 ml of sterile distilled water for a few minutes. The mix was then run in a vortex (dilution Stage I / 10^{-1}), from the 10^{-1} dilution reaction tube, 1 ml was pipetted and put in a test tube containing 9 ml of sterile distilled water and then run again in the vortex (dilution stage II / 10^{-2}). Similar dilution procedures were performed up to a 10^{-4} dilution. From the final dilution stage (10^{-4} dilution) a 0.1 ml were taken aseptically using a measuring pipette, and then put into a sterile Petri dish containing solid PDA (potato dextrose agar, Difco) added with chloramphenicol and then spread evenly on a petri dish. Subsequently, the media containing dilution were incubated at room temperature (25^{o}C - 27^{o}C) for 5 days. In order to obtain pure cultures of the fungus, a purification procedure was conducted at the next stage. Numbers of *Trichoderma* colonies that grow on the dilution of 10^{-1} to 10^{-2} are in a very large number causing it difficult to separate, purification only made in the colonies that grow on the 10^{-4} dilution. Purification is carried out by moving the fungal colony using a sterile needle loop to a new sterile PDA solid media.

Characterization of *Trichoderma.*

After the purification process, each colony of *Trichoderma* isolates was taken using a 0.5 cm bore cores then placed in the middle of new sterile solid PDA and incubated at room temperature (25°C - 27°C) for further used in macroscopic and microscopic observation. Identification of *Trichoderma* done by macroscopic and microscopic observation. The macroscopic identification was conducted by observing the morphology, colony growth pattern, density, color, mycelia zoning, and the diameter of *Trichoderma* colonies grown in the sterile solid PDA medium. Microscopically, the identification was performed by using a microscope observed at the age of 3 days.

For the microscopic observation, the procedure stages started with cleaning the the object glass with 70% alcohol previously before few drops of Lactophenol-Cotton Blue solution was given at the center of the glass object. A part of *Trichoderma* culture was taken aseptically using a loopful then placed on the glass object that has been spilled with the Lactophenol-Cotton Blue and subsequently covered with cover glass and observed under a microscope. Microscopic observations were made using a light microscope (Trinokuler Zeiss Primo Star iLED, 400x) and fungal colonies were identified by observing the conidia, conidiophores and phyalid described by Barnett and Hunter (1978).

Assessment of seedling growth enhancement ability.

To evaluate the effect of *Trichoderma* isolates on seed germination and seedling growth, three isolates (Maros, Takalar and Jeneponto) were inoculated on maize seed. Inoculation was done by soaking with approximately $1x10^6$ spores per ml. One hundred maize seeds soaked in with 10 ml of *Trichoderma* spore suspension then planted in planting box and placed in green house. Percentage of seed germination was counted at 48, 72 and 96 h after incubation. 10 days after inoculation, plant response parameters such as shoot length, root length, dry root weight, dry shoot weight were measured in 10 plants after uprooting the plants and washing them under running tap water to remove residual soil from the roots.

Statistical Analysis

The experimental design used was a completely randomized design (CRD). Each isolate was repeated four times (400 seed/treatment), therefore there were 16 boxes of planting in total including controls. Means were analyzed by analysis of variance (ANOVA) and LSD test at 5% significant level.

3. Results

Trichoderma was found in all samples collected from Maize rhizosphere of Maros, Takalar, and Jeneponto regencies. The fungal isolates have colonies with thick and velvety texture and dark green color. The colonies of all origin grew covering the media. Upper and lower surface show a dark green color with a ring pattern evident in green (Table 1).

Table 1: Cultural characteristics of *Trichoderma* indigenous strains on PDA medium isolated from rhizosphere of Maize growing in dry land of Maros, Takalar and Jeneponto regencies

Trichoderma Origine	Colony character				
	Texture	Density	Surface color	Reverse color	Zonation
Maros	Thick and velvety	Media	Dark green	Colorless	Concentric zones with green on first and third zones and white on second zone
Takalar	Thick and velvety	Media	Dark green	Hyaline with whiteish at the centre	Concentric zones with green on first and third zones and white on second zone
Jeneponto	Thick and velvety	Media	Dark green and white	Hyaline with dark green at the centre	Concentric zone with mix of green and white on first zone, white on second zone and green on third zone

Twenty four hours after inoculation, all isolates formed colonies with smooth colorless misellia and had colony diameter of 40 mm. At 48 hours, the misellia began to thicken as cotton and white misellia and colony size increased up to 60 mm. Colony showed a radial growth pattern with a concentric rings of green and white color. All isolates formed conidia, conidiophore, and phyalid. Both isolates from Maros and Takalar showed subglobose conidia shaped, slightly conidiophores branches formed, phyalid shaped of flask and ampulliform. Jeneponto isolate had subglobose form of conidia, conidiophores with many branch, phyalid shape of flask, ampulliform and lageniform (Table 2 and Figure 1c). At 96 hour after inoculation, the misellia growing thick and coarse grainy, and the white color slowly turn into green. The rapid grow of colonies was observed after 5 days where the diameter of the colony reached environ 90 mm. In this phase the misselia were abundant, thick and velvety and coverred the plate (Figure 1a and 1b).

Tabel 2: Microscopical characteristics of *Trichoderma* indigenous strains grew on PDA medium isolated from rhizosphere of maize growing in dry land of Maros, Takalar and Jeneponto regencies

Observation	**Isolates**		
	Maros	**Takalar**	**Jeneponto**
Conidia			
Shape	Subglobose	Subglobose	Subglobose
Color	Green	Green	Green
Surface	Smooth	Smooth	Smooth
Conidiophores			
Surface	Smooth	Smooth	Smooth
Color	Hyaline	Hyaline	Hyaline
Branching	Few	Few	Plenty
Phialide			
Shape	Flask, ampulliform	Flask, ampulliform	Flask,ampulliform, lageniform
Color	Hyaline	Hyaline	Hyaline
Surface	Smooth	Smooth	Smooth

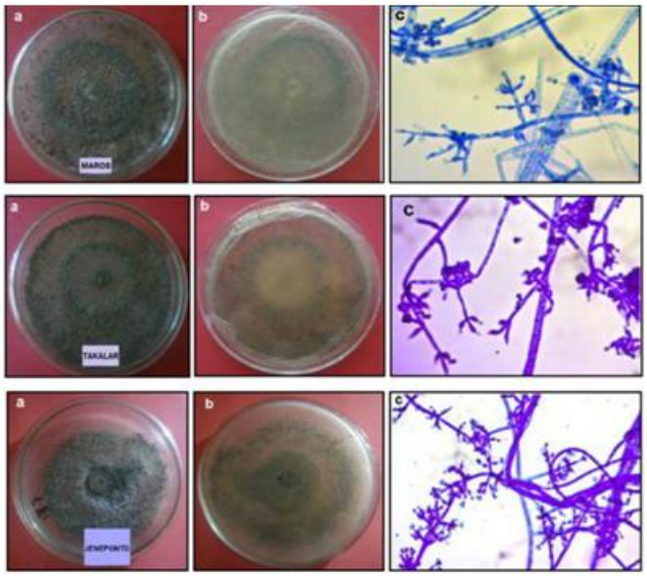


Figure 1: The colony of seven days *Trichoderma* isolates growing on PDA media (a); 'Reverse of colony' of seven days old *Trichoderma* isolates growing on PDA media (b); Microscopic morphology of four days old *Trichoderma* isolates (400 x) (c).

No impact was observed within 48, 72 and 96 hours of incubation on maize seed germination after inoculation by *Trichoderma* of Maros, Takalar and Jeneponto isolates (Figure 2). While, these three isolates of *Trichoderma* treatment affect significantly root length, shoot length, dry root weight, and dry shoot weight of 10 days old seedling. The increase of four parameters above by Maros isolate compared to control was respectively 24.7%, 20.0%, 60.0%, and 57.1%, by Takalar isolates was respectively 16.7%, 17.4%, 60,0% and 42.9%, and by Jeneponto isolate was respectively 40.8%, 17.7%, 80.0%, and 42.9%. Root length treated by Jeneponto isolate was significantly different with that treated by Takalar isolate (Figure 3).

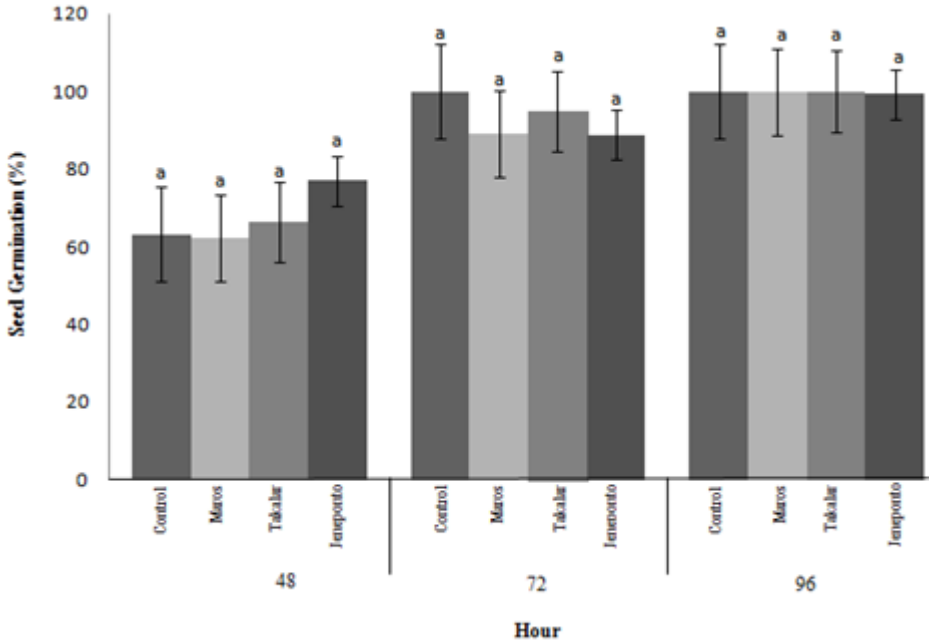
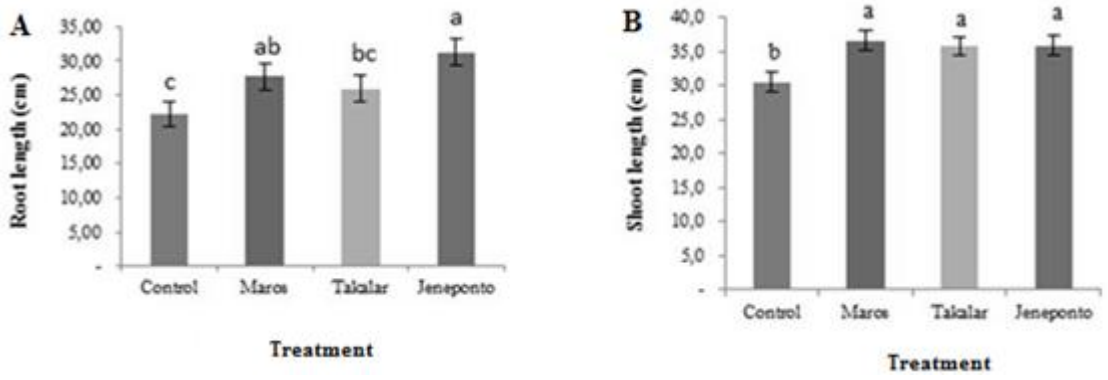


Figure 2: Percentage of maize seed germination at 48 hours, 72 hours, and 96 hours after treatment by *Trichoderma* of Maros, Takalar, and Jeneponto isolates through seed soaking . Means followed with the same letter were not significantly different (P≤ 0.05) according to LSD test.



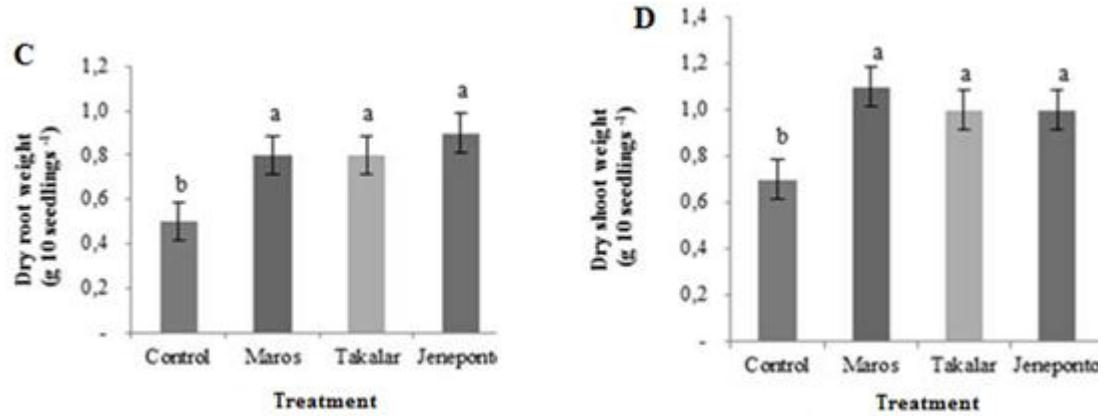


Figure 3. Root length (A), shoot length (B), dry root weight (C) and dry shoot weight (D) of ten days maize seedling after treatment by Trichoderma of Maros, Takalar, Jeneponto isolates through seed soaking. Means followed with the same letter were not significantly different ($P \leq 0.05$) according to LSD test.

4. Discussion

As *Trichoderma* has living habitat in agricultural lands and forests make the *Trichoderma* can be isolated from almost all agricultural land (Choi et al., 2003; Kredics et al., 2014). We also found *Trichoderma* isolates in all soil samples of maize rhizosphere from Maros, Takalar, and Jeneponto regencies. Cultural and morphological characteristic of Maros and Takalar isolates showed the similitude, while Jeneponto isolates indicated the difference. It mean apparently that isolates of Maros and Takalar have same species and isolate of Jeneponto classified in other species.

Maize seed germination was not affected by the application *Trichoderma* isolates. This finding is consistent with the results of Azarmi et al. (2011), Celar and Valic (2005) and Hajieghrari (2010).. This may be attributed to the utilization of existing food reserves in seeds such as carbohydrates, proteins, fats, amino acids and growth hormone to germinate. Seed germination is a process of morphological and biochemical changes that are influenced by the absorption of water, the chemical composition of seeds and phytohormones (Bewley and Black, 1985; Copeland and McDonald, 1995). Therefore, seed germination was not influenced by external factor including *Trichoderma* treatment.

All growth parameters of maize plant includes plant height, root length, shoot dry weight and root dry weight treated by three isolates of Trichoderma were significantly different compared to the control plants (Table 3). These increased growth response of plants depended mainly on the ability of *Trichoderma* to survive and develop in the rhizosphere (Harman et al., 2004). The rhizosphere is among the common ecological niches for *Trichoderma* spp. and provides opportunities for both biotrophy and saprotrophy on plant root exudates (Druzhinina et al., 2011). The increasing root biomass by *Trichoderma*, may be due to auxine-mediated response pathways and also be mediated by a decrease in the level of the plant hormone ethylene (Wang et al., 2002; Contreras-Cornejo et al., 2009). In case of *T.asperelloides* T203, this species have α-1-aminocyclopropane-1-carboxylate (ACC) deaminase gene (*acc1*) that encodes an enzyme which cleaves ACC that is expressed during the interaction with roots of *Brassica napus.* ACC is a key intermediate in ethylene biosynthesis and acc1 enzyme offers a mechanism for facilitating the formation of longer roots (Viterbo et al., 2010). In addition, the *Trichoderma* genomes contain many genes that encode nitrilase (Kubicek et al., 2011). These nitrilases may have a role either in hydrolysing β-cyano-l-alanine, a metabolite which is formed from cyanide released during the final step of ethylene biosynthesis, or in converting the plant metabolite indole-3-acetonitrile to indole-3-acetic acid (IAA), a hormone that promotes the growth of plant roots (Piotrowski and Volmer, 2006). The formation of longer roots by three isolates of *Trichoderma* permit apparently maize to have more capability in nutrient uptake, resulting in more shoot growth. Comparing to Maros and Takalar isolates, Jeneponto isolate enhance more formation of longer roots and therefore more potential for it use in improving maize vigor.

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