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EFFECT OF *TRICHODERMA* FORMULATED WITH CULTIVATED OYSTER MUSHROOM WASTE TOWARD THE GROWTH AND YIELD OF SHALLOT (*Allium ascalonicum* L.)

Sutarman^{1*}, Tjahjanti PH¹, Prihatiningrum AE¹ and A Miftahurrohmat¹



Sutarman

*Corresponding author email: sutarman@umsida.ac.id

¹Department of Agrotechnology, Universitas Muhammadiyah Sidoarjo, Jl. Raya Candi no. 250, Sidoarjo, Jawa Timur, Indonesia

ABSTRACT

The utilization of oyster mushroom waste as an organic fertilizer for onion cultivation in former rice fields frequently encounters soil acidity problems. This experiment aims to determine the effect of administering *Trichoderma* biofertilizer, formulated with Oyster Mushroom Waste (OMW) on the growth and yield of shallots. The experiment was carried out in a greenhouse setting and a completely randomized design with four replications was employed, each experimental unit having six plants. Seeds were planted in a 5 kg capacity polybag containing sterile planting media soil of pH 4.85. The experimental treatments consisted of three types of *Trichoderma* biofertilizers, each containing a different isolate of the fungi (Tc-Jjr-02, Tc-Pjn-01, and Tc-Clk-02), oyster mushrooms waste fertilizer and the only soil. Each isolates formulated in oyster mushrooms waste through the biofertilizer treatment contained a uniform spore population of 0.375×10^6 CFU.g⁻¹. Data were collected on plant height, number of leaves per plant, fresh and dry mass of stover, number of tillers per plant, tuber weight, tuber diameter, soil pH, and the population of each *Trichoderma* isolate at harvest time. The data were analyzed using a one-way analysis of variance (ANOVA). The significant differences between treatments were determined using the Honest Significant Difference test at the 5% level. Based on the growth and yield, the amount of increase for each treatment was calculated compared to the treatment of oyster mushrooms waste fertilizer. Subsequently, the types of isolate and their phylogenetic classes were determined. The results showed that the *Trichoderma* biofertilizer formulated in OMW affected plant height and number of leaves at 7-28 DAP ($p < 0.05$), fresh weight and dry weight of stover, number of tuber shoot, and tuber weight ($p < 0.01$), tuber diameter ($p < 0.05$), soil pH at six weeks after planting and one week before harvest ($p < 0.05$), and also *Trichoderma* population ($p < 0.05$). These three types of this biofertilizer can increase stover fresh weight of shallot between 13.97-52.05%, dry stover weight 67.76-151.42%, number of tillers 44.75-47.00%, heavy fresh tuber 20.31-28.13%, and tuber diameter 3.13-10.97%. The three isolates of the biofertilizer agent were identical to *Trichoderma asperellum*, where the Tc-Jjr-02 isolate showed the best performance in assisting the growth and production of shallot plants in acidic soils.

Key words: acid soil, growth, oyster mushroom waste, shallots, *Trichoderma* isolates, yield

INTRODUCTION

The potential supply of shallot (*Allium ascalonicum*) nationally in Indonesia can be achieved through the development of dry land shallot production. However, the levels of soil acidity and low soil organic matter contents limit the growth of this crop [1]. This condition often occurs in onions cultivated on former rice fields which is a method of crop rotation. The organic matter content of soil can be increased by the application of organic fertilizer obtained from crop residues, livestock manure, and various types of organic waste. One potential organic waste is the residual biomass of mushroom cultivation, including oyster mushroom waste. Many studies related to the use of agricultural waste have been carried out, but very limited literature has shown the conversion and development of cultivated oyster mushroom waste into fertilizer.

One of the waste materials used in cultivating oyster mushrooms is sawdust, which contains lignocellulose [2]. This indicates that the waste utilized in the cultivation comprises lignocellulose as well as fungal biomass. However, the use of oyster mushroom waste directly as a crop fertilizer has not been effective in providing nutrients for the crops. Therefore its use as a fertilizer is largely inadequate, and so is used for land compaction [3, 4]. This condition also causes additional problems due to the waste pile which serves as the source of pest organisms and plant pathogens [5-6] and its toxic dust also causes human health problems [7]. Subsequently, for oyster mushroom biomass and other organic materials to be effectively utilized as fertilizer, it is necessary to improve their quality for easy absorption of their mineral nutrient by plants.

Trichoderma has been shown to effectively degrade organic agricultural waste materials as part of planting media components [8-10] and also provide a positive effect of nutrients for plants [11, 12]. Furthermore, *T. harzianum* in addition to improving canola crop yields [10], and the fresh weight of cucumbers [13], also increases plant resistance to diseases. *Trichoderma* fungi are also a potential organism used for the degradation of mushroom mycelium biomass as well as lignocellulose and various organic materials that remain in the waste of oyster mushrooms. Conversely, *T. harzianum* produces the chitinase enzyme [14] which can damage the cell walls of oyster mushrooms which are composed of chitin compounds and various enzymes that can increase plant growth capacity [15-16].

Different types of *Trichoderma* often have different characteristics in their ability to decompose organic matter to produce useful extracellular compounds for plant growth including onion plants. Therefore, there is a need to test the potential and

effectiveness of the ability of a biofertilizer formulated from the oyster mushroom cultivation waste. The objective of this experiment, therefore, is to determine the response of shallot (*Allium ascalonicum*) to different formulated isolates of oyster mushroom waste and *Trichoderma* biofertilizer.

MATERIALS AND METHODS

Site and experimental design

The experiment was conducted at the Greenhouse of the Department of Agrotechnology, Universitas Muhammadiyah Sidoarjo, Sidoarjo, East Java, Indonesia at an altitude of 6.1 m above sea level, with a temperature of 28-32°C. A completely randomized design (CRD) was used for the experiment. The five treatments used included soil from former rice fields, soil with oyster mushroom waste fertilizer (Soil+OMW), and soil with *Trichoderma* isolates, which were Tc-Pjn-02 (Soil+OMW-T1), Tc-CLK-01 (Soil+OMW-T2), and Tc-Jjr-02 (Soil+OMW-T3). These treatments were repeated four times for a total of 20 experimental units.

Preparation, planting, and harvesting

The soil used for this experiment was taken from the rice fields of Purworejo Village, in sub-district Ngoro, District of Mojokerto, East Java. These lands apply shifting cultivation year-round with paddy fields before planting shallots. The soil was analyzed and categorized as alfisol with a pH level of 4.8. The soil and oyster mushroom waste obtained from oyster mushroom cultivation sites were sterilized in an autoclave [17] to minimize other microbial contamination, before being inoculated by *Trichoderma* isolates. This uncontaminated soil was then placed in a 5 kg capacity poly bag, while the sterile OMW was prepared as the basic ingredient of *Trichoderma* biofertilizer.

The *Trichoderma* isolates (Microbiology laboratory collection at Muhammadiyah University of Sidoarjo), are shown in Figure 1, and their descriptions are listed in Table 1. The three isolates were grown simultaneously on PDA-chloramphenicol media [18] for 10 days. Each culture was crushed and then dissolved with distilled water until it was homogeneously mixed. The suspension of conidiospores from each isolate was then combined with sterile OMW and thoroughly compounded. During mixing, distilled water was added to allow a homogeneous mixture of the conidiospores and OMW with a spore density of 0.375×10^6 CFU.g⁻¹. Three kinds of biological fertilizers which were a mixture of spores from each isolate of *Trichoderma* and OMW (oyster mushroom waste), OMW fertilizer (without *Trichoderma*), and sterile soil used in this experiment were placed in an open container and incubated for 14 days indoors at a temperature of 28°C. The

amounts of fertilizer and biofertilizer were 150 g polybag⁻¹, respectively. Each experimental unit had 6 plants with one plant per polybag. The onion variety planted was Keta Monca from Bima Regency, West Nusa Tenggara Province [19]. Additionally, healthy tubers with a diameter of 18mm were selected for planting and compound NPK fertilizer was then applied 7 days after planting at the rate of 5 g per polybag.

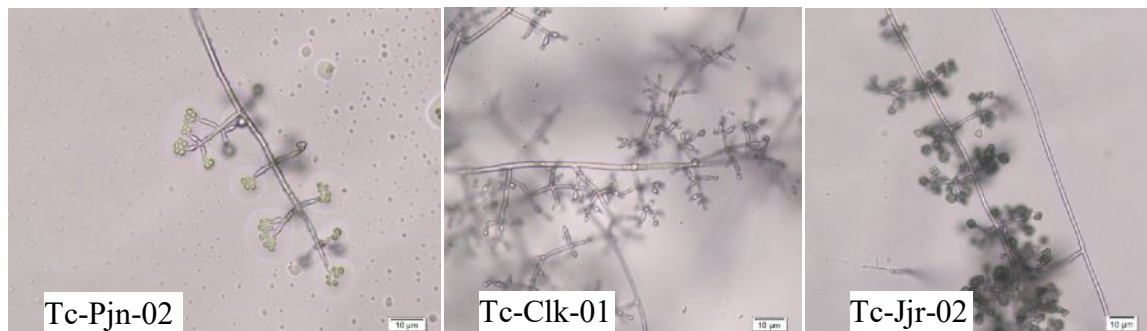


Figure 1: Morphology of the three *Trichoderma*

The data collected from the experiment included the plant height (cm) and number of leaves per plant in a week from 7 to 42 days after planting (DAP); fresh and dry weights of stover at harvest (g), number of tillers, tuber weight (g) and diameter (cm); soil pH at six weeks after planting and one week before harvest; as well as the population of *Trichoderma* (CFU. g⁻¹) at the end of the experiment.

Subsequently, molecular characterization was carried out to ensure the type of *Trichoderma* isolates in the soil. Isolation and DNA preparation up to polymerase chain reaction using primers ITS 1 5'-TCC GTA GGT GAA CCT GCG G-3' (10 pmol) and ITS 4 5'-TCC TCC GCT TAT TGA TAT GC-3' (10 pmol) was done with the procedures of Zhang *et al.* [20]. The PCR products were then sent to a commercial DNA sequence laboratory (1st Base; Singapore). Furthermore, nucleotides were produced from sequencer machines (ABI 3730XL sequencers) and were compared to bank genes using the Basic Local Alignment Search Tool (BLAST) programs available at the National Center for Biotechnology Information (NCBI). The nucleotides of each isolate from ITS 1 and ITS 4 sequences were compared with other sequences in GenBank and analyzed using MEGA X software [21] to obtain a phylogenetic tree.

Statistical analysis

The data obtained were analyzed using the one-way analysis of variance (ANOVA) at the 5% (0.05) level to determine the significant differences in the effect of treatments.

RESULTS AND DISCUSSION

Trichoderma biofertilizer formulated in oyster mushroom waste significantly affected the growth of plant height and the number of leaves ($p < 0.05$) especially during the vegetative phase ($p < 0.05$). Growth charts of average plant height (Figure 2) and the number of leaves per plant (Figure 3) levelled off at 36 to 42 DAP. Regarding its genetic potential, the shallot variety used in this experiment peaked its vegetative growth phase between 36-42 DAP, after which it started its reproductive phase with flower formation [19].

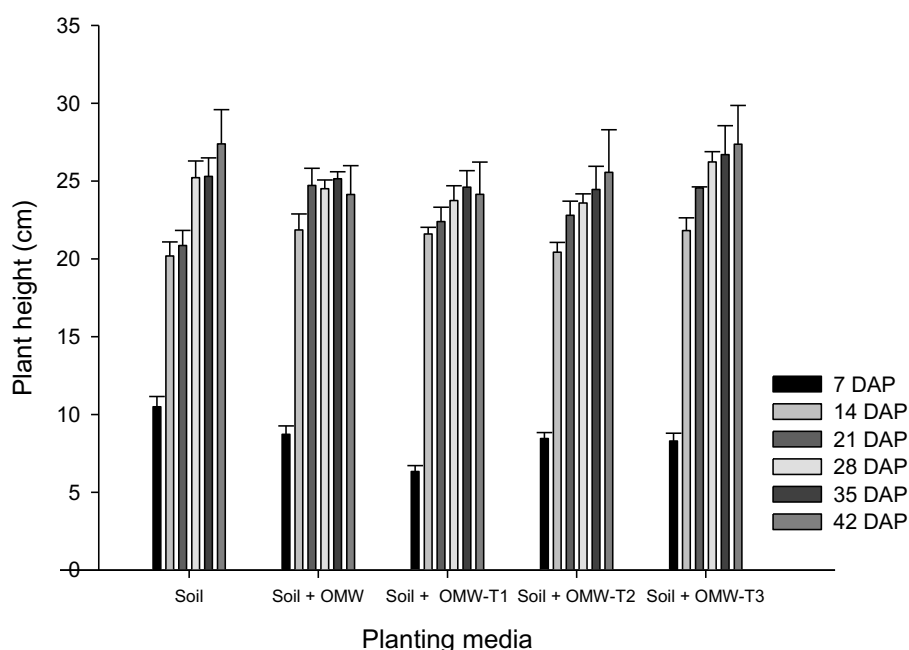


Figure 2: Effect of biofertilizer on plant height (cm) response to shallot plants with different planting media at 7-42 DAP

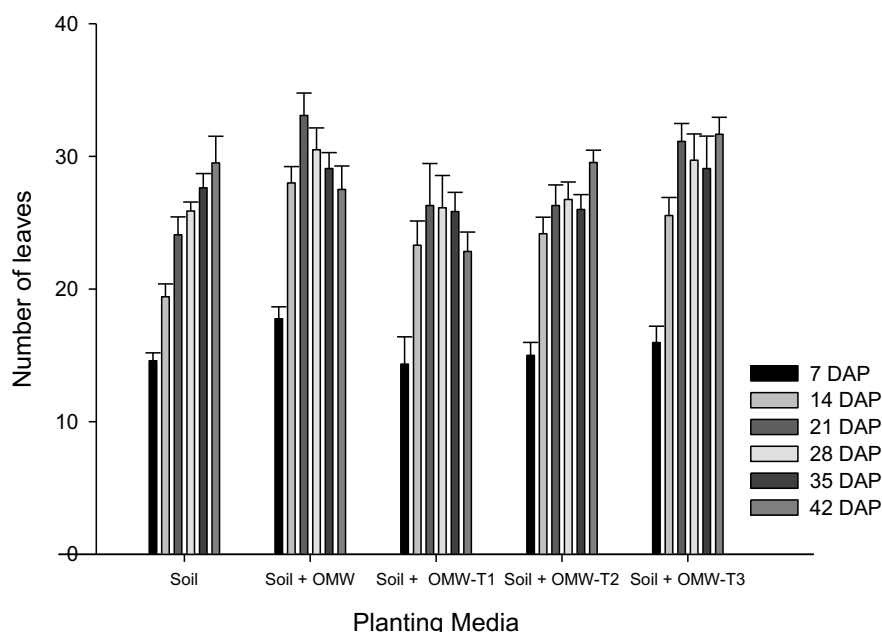


Figure 3: Effect of biofertilizer on the number of leaves per onion plants with different growing media at 7-42 DAP

Biofertilizers were shown to affect the average plant biomass demonstrated by the fresh and dry weights of the stover (Table 2), the number of tuber buds, tuber wet weight, and tuber diameter of the Shallot plant (Table 3). This depicts the addition of nutrients from *Trichoderma* activities as well as OMW fertilizers. Extracellular compounds such as the auxins were produced by *Trichoderma* [11, 22], which is the final result of organic materials decomposition by oyster fungi [2, 23, 24]. The OMW by this biofertilizer is useful for accelerating plant growth [16, 25].

The role of *Trichoderma* in promoting early vegetative growth also continues into the generative phase and the production of plant biomass. In this acidic soil condition, plants that were given this biofertilizer were able to increase the number of tillers by an average of 5.79-5.88; meanwhile based on its genetic potential these plants were able to produce six tillers each [19]. In the treatment of only soil and OMW fertilizer, the number of tillers produced was between 3.25 and 4.00. Conversely, the growing and yield of shallot plants were low at pH conditions less than 5.5 [26]. The optimal soil pH for this plant was 6.0-6.5 [27]. In the treatment without *Trichoderma* having an average initial soil pH of 4.85, the number of tillers per plant at harvest was 3-4, which was well below their optimal potential. Therefore, the characteristic of this biofertilizer in the experiment was its ability to overcome the soil acidic conditions, as there was an increase in its population of about 0.375×10^6 CFU.g⁻¹ to 3.725 - 4.335×10^6 CFU.g⁻¹ (Table 4) at harvest. These

fungi, including *T. harzianum*, were more tolerant and preferred the acidic soil condition of pH 5.5-6.5 compared to pH 6.5-7.5 [28-30]. On the other hand, *Trichoderma* was shown to have increased soil pH from 4.80 at the start of the experiment to 5.88-6.29 one week before harvest (Table 4). The increase in population is consistent with the fact that it helps plants overcome soil acidification [31]. Thus its application as a soil amendment with OMW increases soil productivity agronomically [32]. Therefore, the response of plant roots to biostimulants produced by *Trichoderma* in the form of auxin rhizosphere, peptides, and other active metabolites promote root branching and absorption capacity increases the growth and yield of horticultural crops [33].

The OMW treatment was shown to increase biomass production compared to the control (Table 2). Organic fertilizers have also been shown to enhance plant growth and productivity [34], although the average plant growth rate in this experiment was lower than that of plants treated with biofertilizer. At 36 DAP and one week before harvest, oyster mushrooms waste (OMW) increased soil pH to 6.13 and 5.88, respectively. The sterile deadwood and mycelium fibers, cellulose, protein, and different natural polymer composite fiber materials worked as a buffer against soil acidity [35]. In contrast to the treatment given *Trichoderma*, the microbial activity decreased in soil pH [36] at 36 DAP before harvest.

When viewed from the aspect of the role of increasing plant biomass, crop production, changes in soil pH, and conidiospores populations, there were differences in performance characteristics among the three test isolates. However, based on NCBA's BLAST analysis, it is known that molecularly these three isolates have similarities in their nucleotide sequences. Electrophoresis results of the DNA sequences of the three isolates are shown in Figure 4.

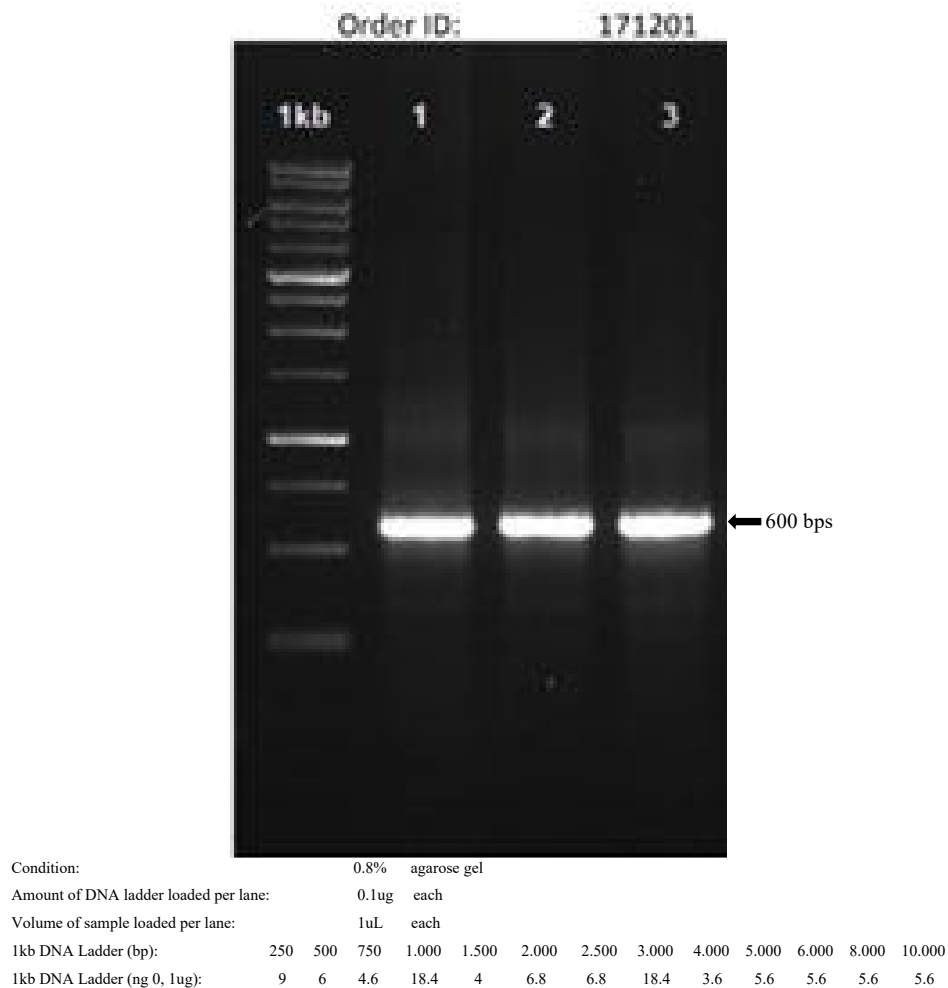


Figure 4: Electrophoresis results of DNA sequences of the three *Trichoderma* isolates; Tc-Pjn-02 (1), Tc-Clk-01 (2), dan Tc-Jjr-02 (3)

Through comparative analysis of the nucleotides of these three *Trichoderma* isolates with ITS 1 and ITS 4 sequences and other sequences in GenBank, a phylogenetic tree was obtained (Figure 5) showing their relationship with various other *Trichoderma* isolates.

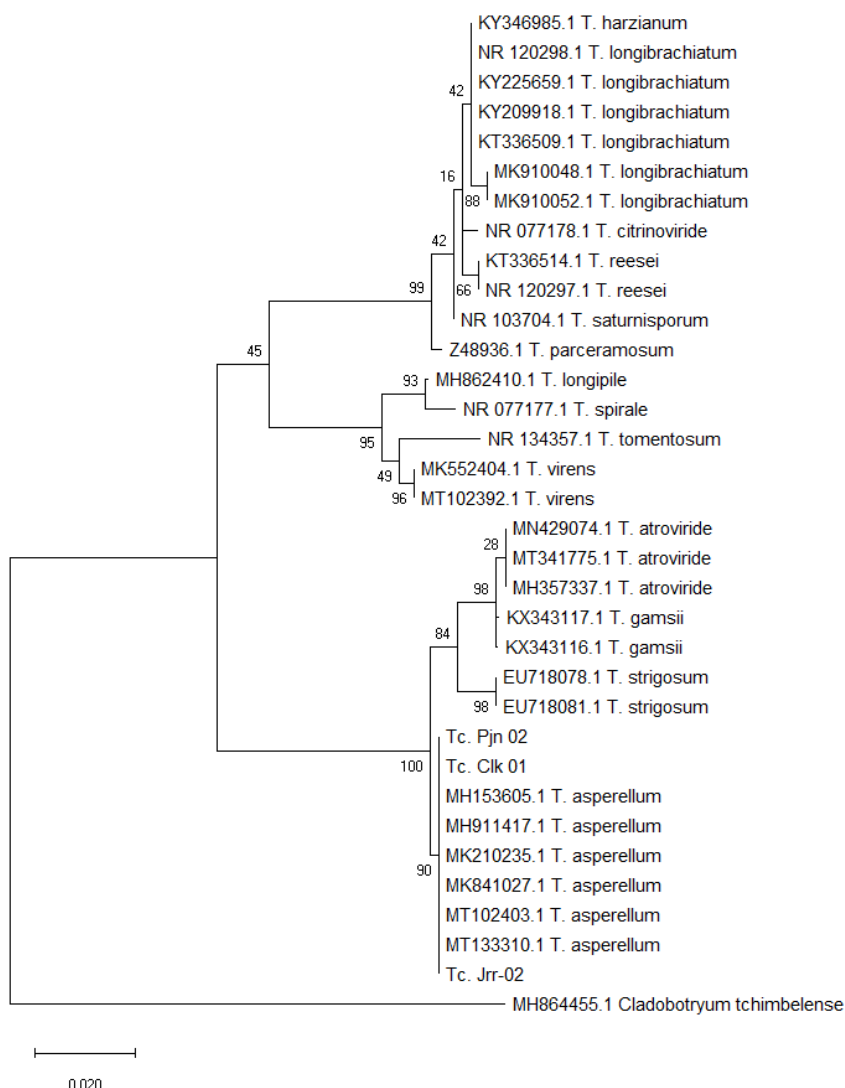


Figure 5: The phylogenetic tree showing three *Trichoderma* isolates (TC-Pjn-02, Tc-Clk-01, Tc-Jjr-02) kinship with various species based on analysis of sequences ITS 1 and ITS 4 with bootstrap 1000x

All three test isolates were identical to *Trichoderma asperellum* with a 100% similarity level [37]. The characteristics of this biofertilizer as shown in this experiment are in line with the performance of its several other isolates that have been tested, to determine their ability to increase growth metabolism and biomass of sorghum-sudangrass [38], maize [39], and *Camellia sinensis* seedlings [40].

CONCLUSION

The *Trichoderma* biological fertilizer formulated with Oyster Mushroom waste showed an increase in plant height and the number of leaves during the vegetative growth phase, as well as an increase in stover fresh weight between 14.0-52.1%, stover dry weight 67.8-151.4%, number of tuber buds 44.8-47.0%, tuber fresh weight 20.31-28.1%, and tuber diameter 3.1-11.0%. It also showed an increase in the soil pH from 4.85 at the beginning of planting, to 4.96-5.54 at 36 DAP, and to 5.88-6.29 one week before harvest. The three isolates of biological fertilizer agents were identical to *Trichoderma asperellum*. However, Tc-Jjr-02 showed the best performance in the growth and production of shallot plants in acid soils.

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Table 1: Morphological description of the three *Trichoderma* isolates

Characteristics	Tc-Pjn-02	Tc-Clk-01	Tc-Jjr-02
Colony (macroscopic)	Coloni is green with white edges	Coloni is whitish-green especially on the edges	Coloni is green with white edges
Microscopic structure	Hyphae are septate, conidiophores of hyaline upright branching and smooth-walled, phialides flask-shaped and thickened with size 5.0-7.0 μm , and conidia subglobose to obovoid green oval measuring 3.2-3.4x2.2-3.0 μm	Hyphae have septa, hyaline conidiophores, upright and irregular, smooth-walled; the flask-shaped Phialides are arranged in pairs with size 5.0-7.0 μm , and conidia are green, oval, smooth-walled, and measures 3.0-3.5x2.0-2.9 μm	The hyphae branched to form an angle, the conidiophores looked hyaline and resembled a pyramid-like shape, the phialides shape looked like a pumpkin with thickened walls measuring 5.0-7.0 μm ; conidia slightly oval or rounded with a slightly greenish color and measuring 2.5-3.5 x 2.0-3.0 μm

Table 2: The means of wet weight and dry weight of stover

Treatment	Wet weight of stover (g)	Improvement compared to Soil+OMW (%)	Dry weight of stover (g)	Improvement compared to Soil+OMW (%)
Soil	19.74 c	21.23 (-)	8.23 c	16.90
Soil+OMW	25.06 bc		7.04 c	
Soil+OMW-T1	30.43 b	21.43	11.81 b	67.76
Soil+OMW-T2	28.56 b	13.97	15.59 a	121.45
Soil+OMW-T3	38.10 a	52.04	17.70 a	151.42
HSD 5%	6.98		2.13	

Means followed by the same letter in the same column are not significantly different ($P>0.05$) by the HSD test

Table 3: The means of number of tillers, tuber weight and tuber diameter

Treatment	Number of tillers	Improvement compared to Soil+OMW (%)	Tuber weight (g)	Improvement compared to Soil+OMW (%)	Tuber diameter (mm)	Improvement compared to Soil+OMW (%)
Soil	3.25 c	18.75 (-)	0.90 c	29.69 (-)	14.75 ab	5.06
Soil+OMW	4.00 b		1.28 b		14.04 ab	
Soil+OMW-T1	5.81 a	45.25	1.54 ab	20.31	13.65 b	2.78 (-)
Soil+OMW-T2	5.79 a	44.75	1.64 a	28.13	14.48 ab	3.13
Soil+OMW-T3	5.88 a	47.00	1.56 a	21.87	15.58 a	10.97
HSD 5%	0.75		0.28		1.59	

Means followed by the same letter in the same column are not significantly different ($P>0.05$) by the HSD test

Table 4: The means of soil pH at six weeks after planting and one week before harvest and *Trichoderma* population in planting media

Treatment	Soil pH at six weeks after planting	The improvement compared to Soil+OMW (%)	Soil pH one week before harvest	The improvement compared to Soil+OMW (%)	<i>Trichoderma</i> population in planting media (CFU.g ⁻¹)
Soil	5.00 b	22.05	4.96 a	15.65	0.0 c
Soil+OMW	6.13 a		5.88 b		0.0 c
Soil+OMW-T1	4.96 b	0.79 (-)	6.17 c	4.93	3.725 x 10 ⁶ b
Soil+OMW-T2	5.29 b	7.09 (-)	5.88 c	0	3.925 x 10 ⁶ ab
Soil+OMW-T3	5.54 b	2.36 (-)	6.29 c	6.97	4.335 x 10 ⁶ a
HSD 5%	0.90		0.46		0.650 x 10 ⁶

Means followed by the same letter in the same column are not significantly different ($P>0.05$) by the HSD test

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