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The Effect of Hot Water Treatment and Dose *Trichoderma* sp. to Plant Tissue of Seedling Growth from Bud Chips of Sugarcane (*Saccharum officinarum*)

Pengaruh Perendaman Air Panas dan Dosis Trichoderma sp. terhadap Kualitas Jaringan pada Pertumbuhan Benih Asal Mata Tunas Tebu (Saccharum officinarum)

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Abstract

Sugarcane (*Saccharum officinarum*) is a high-value economical crops, only grows in tropical climates of Java and parts of Sumatra. Ratoon stunting disease (RSD) is a vascular disease in sugarcane which its visual symptoms is difficult to detect. The disease has spread across the sugarcane plantations in Indonesia with the percentage of attacks 10-100%. The use of buds chips treatment of seedling plant is an act of reducing pathogen development. The research effect of hot water treatment / HWT (0, 30, 60, and 90 min) and dose application of *Trichoderma* sp. (0, 25, and 50 g) using 864 varieties, is designed with a completely randomized factorial design. The results showed that *Trichoderma* sp. able to infect the roots and stems of seedling age 3 months, part of plant tissue increase of protein and proline content, decrease of glucose content so that increase resistance and health in the growth of sugarcane seedling before planting in the land.

Abstrak

Tanaman tebu (*Saccharum officinarum*) adalah tanaman yang bernilai ekonomi tinggi, hanya tumbuh di daerah beriklim tropis terutama di Jawa dan sebagian Sumatra. Penyakit ratoon stunting disease (RSD) merupakan penyakit pembuluh pada tebu yang sulit dideteksi gejala visualnya. Penyakit tersebut telah tersebar di seluruh pertanaman tebu di Indonesia dengan presentase serangan mencapai 10-100%. Penggunaan bahan tanam benih asal mata tunas (bud chip) dapat mengurangi perkembangan patogen. Penelitian pengaruh waktu perendaman air panas (0, 30, 60, dan 90 menit) dan aplikasi dosis jamur *Trichoderma* sp (0, 25, dan 50 g) terhadap pertumbuhan benih tebu klon 864, dirancang dengan menggunakan rancangan acak lengkap faktorial. Hasil penelitian menunjukkan bahwa jamur *Trichoderma* sp mampu menginfeksi bagian akar dan batang benih umur 3 bulan, pada bagian jaringan tanaman terjadi peningkatan kadar protein dan prolin serta penurunan kadar glukosa sehingga meningkatkan ketahanan dan kesehatan pada pertumbuhan awal benih tebu sebelum ditanam di lahan.

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INTRODUCTION

Sugarcane is a group of seasonal plantation plant used as a raw material to make sugar, grew in a tropical climate region, included to grass family and annual plant. On its development to be the raw material in sugar production, sugarcane passes four stages; they are bud, nursery, and ripeness.

The decline of sugarcane bud's health and endurance in a field is caused by ratoon stunting disease (RSD), bud chips spotted and *pokkah bung*. Those diseases are very harmful to the sugarcane ages 2-3 months so its seedling growing is imperfect. A stem cannot be sterilized. The hot water treatment (HWT) to the bud chips is one of ways in slowing the RSD development (Semangun 2000; Anonymous 2011; Anonymous 2013).

Trichoderma sp. is a fungus from Deuteromycetes with straight conidiophores, many branches, cone shape, forming a chlamydospores shape, the colony in its growth grows fast and its color is white until green. The perfect shape of this fungus is generally known as *Hypocreales* or *Eurotiales*, *Clacipitales*, and *Spheriales*. The species included in the same group as *Trichoderma* sp. can show different species in *Hypocrea* as an *anamorf* because there are many sexual shape discrepancies from *Trichoderma* sp. (Chet 1987; Cook & Baker 1989; Alexopoulos et al. 1996; Semangun 2001). *Trichoderma* sp. is proven to be able to control decomposed disease on a vanilla stems (*Fusarium oxysporum* f. sp *vanilla*), the research was done by Hadisutrisno (2000), wilted on tomato caused by *Fusarium oxysporum* (Alfizar et al. 2011), and blight on potato leaves caused by *Phytophthora infestans* (Baihaqi et al. 2013). *Trichoderma* sp. effects the forming of cell wall constituents such as protein, glucose, and proline. Those ingredients have a role in endurance and plant scanning to pathogen.

RSD endemic is a disease which contracted by soil, is very harmful for seedlings at the beginning growth and is considered a usual phenomenon even though it decreases the production until 30-40%. To improve a self-sufficient of sugar in 2014 in order to increase the sugarcane yield until 8,5%, efforts must be done to improve the health and endurance of the seedlings. So, the RSD disease development must be prevented. Therefore this research is done to know the effect of HWT variation toward the tissue quality at the bud chips seedlings growing. The testing is done with related institution hoping to give a contribution in spreading the information through the working units from the government

institution in every regency.

METHODS

The multiplication of *Trichoderma* sp. was done at *Laboratorium Balai Proteksi Tanaman Perkebunan* (Plantation Protection Hall of Laboratory) at Salatiga. The bud chips is taken from *Kebun Benih Lembaga* at Boyolali, the HWT treatment and seedlings multiplication was done at green house Faculty of Agriculture Tunas Pembangunan University Surakarta. The research materials and equipments are including sugarcane seedlings clone 864, *Trichoderma* sp. fungus, soil media, maize flour, grow controller substance, hipochlorit, combining fertilizer (NPK), water heater, polybag, sack, large plastic bucket, and soil strainer.

The experiment was arranged in a completely randomized factorial design, consisted of two factors. The first factor was *Trichoderma* sp. fungus inoculation including without *Trichoderma* sp. inoculation (T0), with *Trichoderma* sp. inoculation 25g (T1), and with *Trichoderma* sp. inoculation 50g (T2). The second factor was Hot Water Treatment consisting of treatment without hot water (P0), heating treatment for 30 minute (P1), heating treatment for 60 minute (P2), and heating treatment for 90 minute (P3). Each treatment was repeated three times and each replicate consisted of 10 plants.

The experiment was conducted to detect the resistance and the health of the plant consisting of protein level, glucose level, proline level, death of plants, the disseminating of *Trichoderma* sp. on its stems and root. Then, the data analysis were observed visually and analyzed with ANOVA, the Duncan Test was also used with the significance of 5 %.

RESULTS AND DISCUSSION

The detection of the resistance and the health of the plant is based on the parameter of the protein level, glucose level, and proline level. Figure 1 shows that the sugarcane seedling without the *Trichoderma* sp. inoculation, its protein level is lower than that sugarcane seedling with inoculation and it shows insignificant different in the hot water treatment variation (Table 1). *Trichoderma* sp., besides having a role as the pathogen controller, declining or failing the tomato yields, and helping to increase the phosphor (P element) availability which is difficult to get from the nature, it also is having a role as a soil decomposer. The availability of the P element is needed by the plants when they are growing and the in-

creasing of the yields are effected by this element also. Rosmarkam and Yuwono (2002) state that the P element is involved in forming a number of certain proteins which are important in the photosynthesis and respiratory as a result, the P element is important in the entire plants growing. Besides, it also is essential to repair the plant root system.

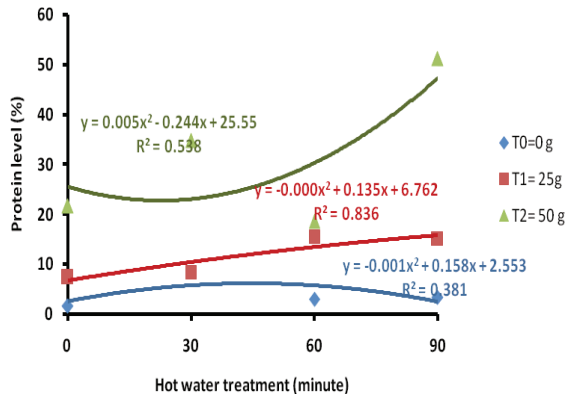


Figure 1. The influence of hot water treatment and *Trichoderma* sp. inoculation toward the protein level.

The result of the test shows that giving *Trichoderma* sp. affects on the growth of sugarcane seedling (the height of the plant, the length of the leaves, the amount of the leaves, the length of the root, and the volume of the root). Those are occurred because the fungal hypha of *Trichoderma* sp. in the root keeps the proteins in order to be used in physiology process. The proteins are formed from the nutrients such as C, H, O, N, S, P, and K which, in the synthesis process, will be transformed into nucleic acids, growth hormone and enzyme inside the plants body, and finally the proteins have a role in a daily cell activities such as the cleavage process or cell replacement, the change of the broken or old cell (Parman, 2007).

Living creature need proteins in their live used to catalyze a reaction, if as an enzyme, microtubule protein and microfilaments protein in the ribosome have a structural function, not as a catalyze. The other function are as an electron transporter during the photosynthesis and respiratory process, it is used also as an amino acids back up in the seedling germination (Cech & Bass 1986). Proteins consist of one or more polypepti-

Table 1. The protein, glucose, and proline level on the sugarcane seed age 3 months

Treatment	Parameter		
	Protein Level (%)	Glucose Level (%)	Proline Level ($\mu\text{mol g}^{-1}$)
<i>Trichoderma</i> sp. (T)			
T ₀	3,30 ^a	3,30 ^a	1,66 ^a
T ₁	1,36 ^a	1,36 ^a	5,17 ^b
T ₂	1,03 ^a	1,03 ^a	4,79 ^b
<i>Hot Water Treatment</i> (P)			
P ₀	3,36 ^a	3,36 ^a	3,26 ^b
P ₁	1,51 ^a	1,51 ^a	4,53 ^c
P ₂	1,40 ^a	1,40 ^a	1,85 ^a
P ₃	1,32 ^a	1,32 ^a	5,84 ^c
Interaction between <i>Trichoderma</i> sp. & <i>Hot Water Treatment</i> (TXP)			
T ₀ P ₀	1,65 ^a	6,86 ^b	2,2709 ^b
T ₀ P ₁	8,46 ^b	6,99 ^b	3,3206 ^b
T ₀ P ₂	2,97 ^a	5,70 ^b	0,7298 ^{ab}
T ₁ P ₀	3,38 ^a	5,84 ^b	5,3978 ^b
T ₁ P ₁	8,45 ^b	3,80 ^a	5,7328 ^b
T ₁ P ₂	15,56 ^{cd}	3,21 ^a	1,4222 ^{ab}
T ₁ P ₃	15,22 ^c	3,21 ^a	8,1226 ^b
T ₂ P ₀	21,64 ^e	3,80 ^a	2,1146 ^b
T ₂ P ₁	34,83 ^f	3,10 ^a	4,5491 ^b
T ₂ P ₂	18,60 ^{de}	3,08 ^a	3,3877 ^b
T ₂ P ₃	51,22 ^g	2,37 ^a	9,1053 ^b

Note: Score followed by the same letter in the coloum shows insignificant different based on the Duncan Test 5% rank.

de chain and each consists of hundreds of amino acids, the type and the amount of amino acids subunit effect the composition and the size of each protein in most of plants protein the weight of the molecule is more than 40.000 daltons (ferredoxin protein in the photosynthesis process). As a result, the protein improves the tenacity of the plants and it agrees with the research result which (Figure 1) that is the increase of *Trichoderma* sp. dose can improve the protein level.

The analysis result in the Figure 2 above shows that the seedling with *Trichoderma* sp. inoculation has lower glucose level than that of the seedling without *Trichoderma* sp. inoculation and it shows insignificant in the hot water treatment (Table 1). The lysis mechanism on the pathogen of hypha is marked by the color changing on the pathogen of hypha becoming crystal clear and empty the cell content is used by the biological agents as a nutrient and the ability of the biological agents produces enzyme that can lyse the pathogen cell wall. It agrees with Djarir statement (1993) who states that *Trichoderma* can produce antibiotic that prevents the growth of the pathogen of hypha. For example, *Trichoderma viridae* produces gliotoxin and viridian antibiotic, while *Trichoderma* sp. can produce β -1 enzyme, 3 glucanase and chitinase which can lyse the pathogen of hypha. The resulted enzyme can destroy the fungus pathogen cell wall and will cause cell death.

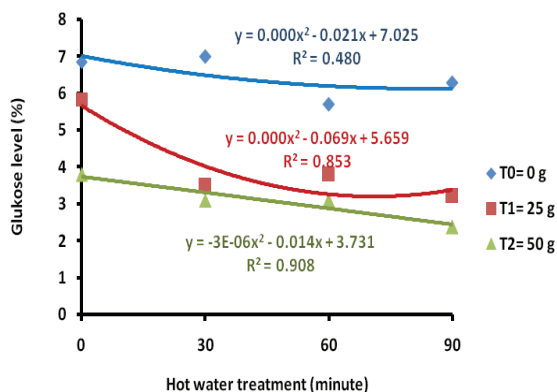


Figure 2. The influence of hot water treatment and *Trichoderma* sp. inoculation toward the glucose level.

The antagonist fungus has been known since long time ago as a bio control agents which act through the production of gliophirin and gliotoxin (Howell on Suwandi 2008). The parasitic produces hydrolysis enzyme such as 1, 6- β glucanase (Djonovic et al. on Suwandi 2008), competes to Fe nutrient by secreting siderofor (Wilhite et al. on Suwandi 2008) and also scans the tenacity (Vi-

terbo et al. on Suwandi 2008). In this research, the domination on each mechanism of declining the disease cannot be reviewed in depth (Suwandi 2008).

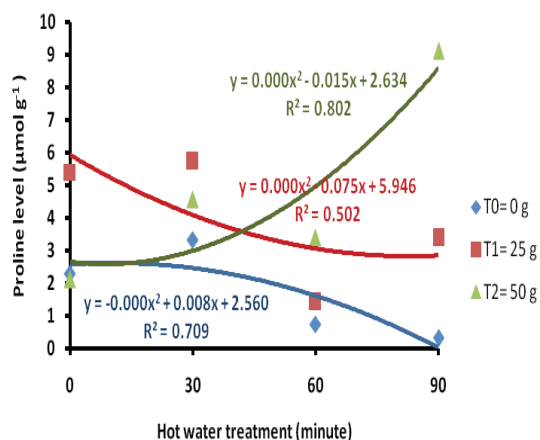


Figure 3. The influence of Hot water treatment and *Trichoderma* sp. inoculation toward the proline level.

The seedling without inoculation has higher proline level than that of with the inoculation, because the proline level is one of the indicator on the occurrence of plants drought (Maestri et al. 1995; Farahani et al. 2008; Johari-Pireivatlou et al. 2010). Johari-Pireivatlou et al. (2010) says that proline can reduce the occurrence of protein damage which has an important role in the growth. BNR inoculation is not causing the proline exalation so that the abiotic disease intensity by the drought occurrence is low.

The adaptation mechanism done by the plants by adjusting the osmotic cell pressure forms an organic compound accumulation, so it reduces the potential of water inside the cell without limiting the enzyme function and keep the turgor cell. The low of the saturated soil becomes a barrier factor to the nutrient transport to the surface of the root in a drought condition (Lestari 2006). According to Christine et al. (1996) and Sasli (1999) the seedling of cotton plant and cocoa inoculated with mycorrhiza fungus has low proline level if the water level is decline and the decline reaches 70,33%.

Figure 4 shows that the increase on the hot water treatment variation to the *Trichoderma* sp. inoculated seedling and without inoculation shows insignificant different in the plant death. The increase of the time lapse treatment in the inoculated seedling does not cause the plant death (0%), while the seedling which is not being inoculated and having no treatment, the death reaches 5% (Table 2). *Trichoderma* sp. fungus improves

the endurance and the health of the plants so the inoculated plants does not have the cell damage and the death.

Table 2. The effect of hot water treatment and *Trichoderma* sp. inoculation toward the plant death.

Treatments	Parameter	
	Death of Plant (%)	
<i>Trichoderma</i> sp. (T)		
T0	1,11	a
T1	0,14	a
T2	0	a
Hot water treatment (P)		
P0	0,74	a
P1	0,37	a
P2	0,37	a
P3	0,19	a
Interaction T x P		
T0 P0	5	ab
T0 P1	3,33	ab
T0 P2	3,33	ab
T0 P3	1,67	ab
T1 P0	1,67	ab
T1 P1	0	a
T1 P2	0	a
T1 P3	0	a
T2 P0	0	a
T2 P1	0	a
T2 P2	0	a
T2 P3	0	a

Note: Score followed by the same letter in the row shows insignificant different based on the Duncan Test 5% rank.

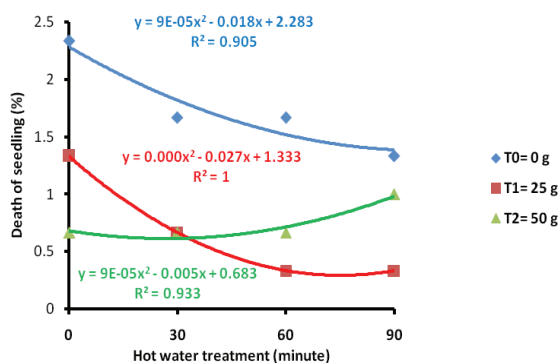


Figure 4. The influence of hot water treatment and *Trichoderma* sp. inoculation toward the plant nuisance

The result of Habazar and Yaherwandi

research (2006) on the biological control using *Trichoderma* shows that paracite hypha of *Trichoderma* will grow abreast with the pathogen hypha and form side branches like a hook in the hypha surrounding and are able to penetrate the hypha pathogen. *Trichoderma* sp. hypha can grow and form a conidia inside of the pathogen hypha even it can penetrate to the rest structure of pathogen such as sclerotia (Sunarwati & Yoza 2010).

Enrich soil with soil microbe can suppress the development of plant disease caused by soil pathogen. The use of soil microbe in planting helps the supplying of nitrogen (N), phosphor (P), calium (K) so it can improve the quality of the plant (Setyowati *et al.* 2003). Microbe given with the organic compound also can improve the quality of soil aggregation (Rahimi 2000). The result of Setyowati *et al* research (2003) *Trichoderma* fungus is able to decrease the rotten root disease and the growth of weeds on the lettuce.

Table 3. The influence of hot water treatment and *Trichoderma* sp. Inoculation toward the root and stalk infection

Treat-ments	<i>Trichoderma</i> sp. Infection	
	Root (%)	Stem (%)
<i>Trichoderma</i> sp. (T)		
T0	0	0
T1	32,78	32,78
T2	33,06	33,06
Hot water treatment (P)		
P0	21,11	21,48
P1	22,22	21,85
P2	22,22	22,22
P3	22,22	22,22
Interaction T x P		
T0 P0	0	0
T0 P1	0	0
T0 P2	0	0
T0 P3	0	0
T1 P0	93,33	96,67
T1 P1	100	96,67
T1 P2	100	100
T1 P3	100	100
T2 P0	96,67	96,67
T2 P1	100	100
T2 P2	100	100
T2 P3	100	100

Note: Score followed by the same letter in the columns shows insignificant different based on the Duncan Test 5% rank.

Table 3 shows that fungus interaction with *Trichoderma* sp. treatment and hot water treatment shows a significant different between inoculated *Trichoderma* sp. seed and non-inoculated *Trichoderma* sp. seed.

Figure 5 and 6 shows that the root without *Trichoderma* sp. inoculation has no infection, while the inoculated root with *Trichoderma* sp. has 100% infection in the root area in the 30, 60, and 90 minute of treatment.

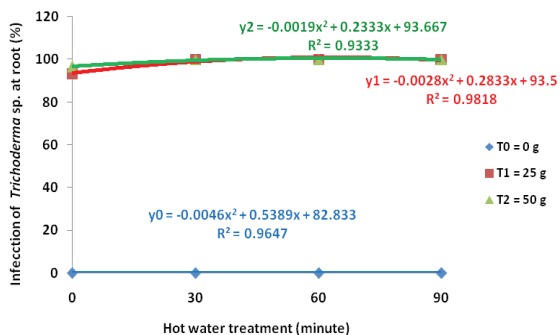


Figure 5. The influence of hot water treatment and *Trichoderma* sp. inoculation toward the *Trichoderma* sp. root infection.

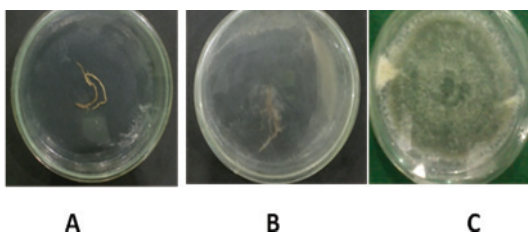


Figure 6. Infection of *Trichoderma* sp. on the root macroscopically dan microscopically on the day 2 incubation (A: root without inoculation; B: root with inoculation)

The character of high speed of growing on the *Trichoderma* sp. and *Trichoderma virens* is one of the important factors which determine the potential of biological agents (Sunarwati & Yoza 2010). The important factor determining the antagonist microorganism activity which can control the pathogen is having the high speed of growing so that it can compete with the pathogen in case of food and space control and finally it can suppress the growth of fungus pathogen (Djafaruddin 2000; Sunarwati & Yoza 2010).

Figure 7 and 8 shows that without *Trichoderma* sp. inoculation, the stems area has no infection (0%), while the stems area which is being inoculated by *Trichoderma* sp. 1 day inoculation (B) and 7 days inoculation (C), the stems area

is 100% free from infection in all hot water treatment variation. Djafaruddin (2000) explains that *Trichoderma* sp. has an important nature as the biological controller that is it can grow fast in a various substrate and have a competition ability either in getting food or in having grow space. Supported by Habazar and Yaherwandi (2006), the ability of *Trichoderma* in preventing the growth of fungus pathogen often is related with the ability in producing chitinase. This enzyme causes a damage in the pathogen fungus cell which can cause cell death.

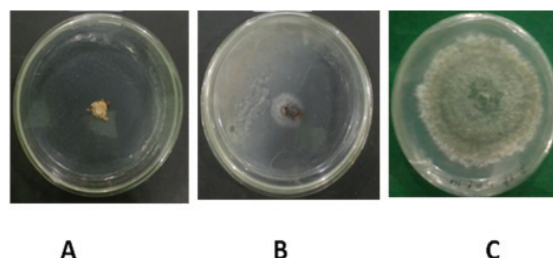


Figure 7. The influence of hot water treatment and *Trichoderma* sp. inoculation toward the *Trichoderma* sp. stalk infection.

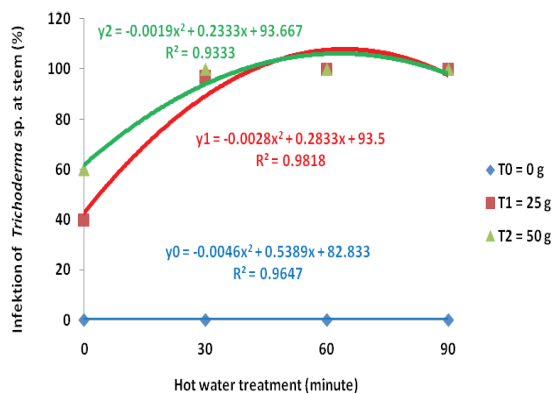


Figure 8. Infection of *Trichoderma* sp. on the stalk macroscopically dan microscopically on the day 2 incubation (A: stalk without inoculation; B: inoculated stalk age 1 day, C: inoculated stalk age 7 days)

CONCLUSION

The inoculation of *Trichoderma* sp. and variation of hot water treatment in the bud chips of sugarcane is able to improve the protein level, proline level, and to decrease the glucose level. It also improves the tenacity and the health of sugarcane seedling preliminary growth. *Trichoderma* sp. is able to infect the root and the sugarcane stems.

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