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The Growth of Root Rot Disease on Pepper Seed Applied by *Trichoderma Harzianum* Inoculum

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Abstract *- Root rot disease on pepper caused by Phytophthora capsici is one of the most important diseases on pepper. The using of antagonistic fungus of Trichoderma harzianum as a biological control agent of the pathogen is one of the important alternatives in controlling P. capsici without causing negative effects on the environment. The objectives of the research were to study about the ability of T. harzianum inoculum application in inhibiting the development of root-rot disease, influenced the growth of pepper seed, to studythe effective length time application of T. harzianum inoculum in inhibiting the development of root rot disease, and increased the growth of pepper seedlings. This research was arranged in a completely randomized design, with five treatments of length time application of T. harzianum inoculum i.e. control treatment without applicationtime of T. harzianum inoculum (K), application time of T. harzianum inoculum for 0 week (S0), application time of T. harzianum inoculum for 1 week (S1), application time of T. harzianum inoculum for two weeks (S2), application time of T. harzianum inoculum for three weeks (S3), and application time of T. harzianum inoculum for 4 weeks (S4) before planting. Each treatment was repeated15 times. The observed parameterswere disease percentage, the inhibition of antagonistic fungus, disease infection rate, plant height, number of leaves, wet and dry weight of plant, stem and leaves on pepper seed, and P. capsici population density. The result showed that application time of T. harzianum inoculumfor 4 weeks (S4) before planting is the most effective time in inhibiting the development of root rot disease than the other treatment sand also had significant effect on increasing the growth of pepper seed. The antagonism test showed that T. harzianum could inhibit P. capsiciin vitro. This result proves that application time of T. harzianum inoculums for 4 weeks (S4) before planting is more effective in inhibiting the development of root rot disease and able to increase the growth of pepper seed.***

Keywords *— Root rot disease; Trichoderma harzianum; pepper seed; affecting factors; plastics injection molding; repeatability; test result*

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I. INTRODUCTION

A solution to overcome root rot disease on pepper is important to be found. Nowadays, *Phytophthora capsici,* a root rot pathogen fungus is not only found in Bangka and Lampung but it also spread over the pepper plants in Java, Kalimantan and Sulawesi. This fungus can cause death on pepper plants within a relatively short time. Kasim (1990) stated that that the damage due to root rot disease attacks reached up to 10-15% per year. *Phytophthora* fungus is easily carried by water, soil or infected plant part so that the fungusmay present in all growing area. If a plant in one area is infected by the disease, the fungi will spread to the surrounding plants within 1-2 months. Some of the characteristics of *P. capsici* are soil borne; difficult to be detected; easily spread through contaminated soil, water and infected plant. The other characteristic is that the zoospore that can move and swim actively through water contained in the soil which makes the fungus is easily to infect all plant's part even the fungus is naturally live in the soil. Disease transmission through the bottom of the plant can cause the plant die quickly. A wilted plant symptom creates on the ground is an indication that shows over infection in the soil. Infected plant should be destroyed immediately. Purwantisari and Hastuti (2005) stated that prevention action should be taken on pepper plants located around the infected plant to prevent contamination.

A treatment using biological agent such as antagonist fungus *Trichoderma* spp. has been widely applied to control the development of the disease (Abdel-Moity *et al.,* 1982; Chet, 1984; Fahim*et al.,* 1989; Abada, 1992). Some success stories of *Trichoderma* application for controlling the diseases development both in green house, nursery or even in the field have been widely reported (Bisiach *et al., 1985,* Gullino, Mezzalama and Garibaldi, 1985; Gullino and Garibaldi, 1988). Several studies showed that *Trichoderma* spp. has antagonistic function against pathogen fungus *Phytophthora infestans* which causes leave and root rot in potato (Purwantisari and Hastuti, 2009) and filtrate application of *Trichoderma* spp can control the growth of pathogen fungus and stimulate the growth of the plants (Harman *et al.,* 2011; Lorito *et al*., 2010. The research conducted by Tindaon, (2008) showed that the combination between pathogenic fungus *T. harzianum* and organic fertilizer could control the growth of pathogenic fungus *Sclerotiumrolfsii*of a soybean.

Trichoderma harzianum from *Trichoderma*spp has been reported for its potential biological agent due to its antagonist characteristic against the soil borne pathogenic fungus. The application of *Trichoderma*spp on soil is micro parasitic and antibiotic which role to inhibit the growth and development of soil borne fungi pathogens (Vinale *et al*., 2008; Rubio et al., 2009; Benitez *et al*., 2004). Pepper seedling on applied soil with *Trichoderma* spp is an initial control or preventive treatment to inhibit the root rot disease as it spread through infected plant. This treatment is expected to produce healthy seedling pepper. According to previous theoretical approach, a research that studies the ability of *Trichoderma harzianum* in inhibiting the root rot disease and affecting the growth of pepper seed should be conducted. The emphasis of the research is the length time application of *T.harzianum* before planting that inhibits the root rot disease and affects the growth of pepper seed.

II. Materials and Methods

The experiment was conducted in Mycology Laboratory, Faculty of Agriculture, Gadjah Mada University and green house in Condong Catur, Yogyakarta. The study was conducted from May 2010 to July 2011 consisted of preliminary and main study. The experiment was designed using completely randomized design with five treatments of length time application of *T. harzianum* inoculum. The treatments were as follows: without application of *T. Harzianum* as control (K), application time of *T. harzianum* inoculum for 1 week (S1), application time of *T. harzianum* inoculum for 2 weeks (S2), application time of *T. harzianum* inoculum for 3 weeks (S3), and application time of *T. harzianum* inoculum for 4 weeks (S4) before planting. Each treatment was repeated 15 times. The result of the experiment was tabulated, statically analyzed, and tested using analysis of variance at 5% significance level. DMRT test was applied in order to determine the significance level among treatments.

The observed parameters in preliminary study were the symptom and growth of root rot disease in pepper seed obtained from Experimental Field of Spice and Industrial Plants Research (Balittri), Sukabumi regency. These observations were conducted due to the presence of root rot disease prior inoculum application which caused the studied plants die during the distribution to the study site. The observed parameters during main study were in vitro test on the inhibition ability of antagonist fungus against pathogenic fungus (Fokema, 1976), the percentage of infected plants, disease infection rate, measurement of height and counting the number of leaves, wet and dry weight of plant, and the density of *P. capsici* in the soil.

III. Results and Discussion

Based on the field survey that identified the symptom of pepper disease, root rot found in the field is shown in Fig. 2(a). The whole plant was withered with yellowish leave and experienced decreased turgor for further symptom (Fig. 2a), black spot on the center and the edge of the leave showing infections of the disease (Fig. 2b). The black spot occurred at the edge of the leaves looks serrated and noticeable during the fresh symptom, but when the leave starts to dry the leave will withered and fall as shown in Fig 2b.

a b

Figure 2. The pepper root rot symptom found in the field (a) the plant was withered with yellowish leave, (b) black Spot in the leave

The isolated fungus that cause pepper root rot obtained from soil samples of infected plants in Wonosari using V-8 medium to produce *P. capsici* isolate was not succeed for propagation purposes due to contamination. Based on laboratory identification, the microscopic sporangium fungus showed oval shape with papilla was obviously shown in the tip of the sporangium (Fig. 3). This feature shows morphology characteristic of *P. capsici* that causes root rot on pepper.

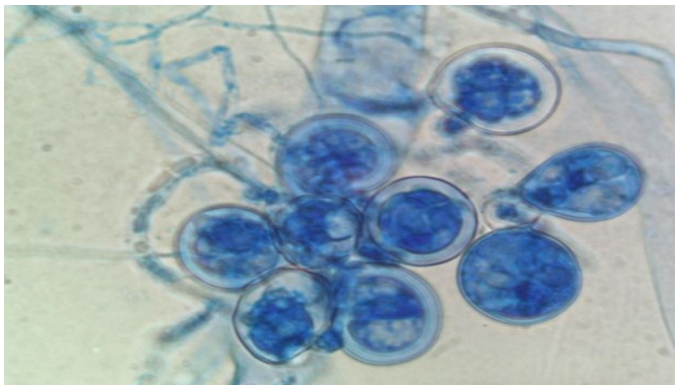


Figure 3. The isolate of *P. capsici* sporangium Indirectly, sporangium can germinate to form zoospores which roles as important inoculum on stem rot disease transmission (Abada, 1994: Semangun, 2000).

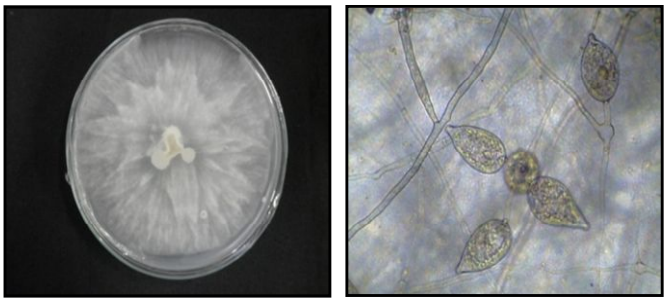
Pepper seeds that were used in this preliminary experiment were 100 plants obtained from Balittri, Sukabumi regency. The plants showed disease symptom at the time the pepper seeds were transferred into the study site, therefore the researcher conducted observation to observe the symptom and the development of root rot disease on the pepper. Based on the observation, the disease occurred on the plants was root rot symptom and could cause death within a short time. The mortality percentage on pepper seed is shown in Table 1.

Table1.The mortality percentage of pepper seed

Observation at week	The mortality percentage of pepper seed
I	6
II	9
III	14
IV	21
V	35
VI	54
VII	76

Table 1 shows increased disease of pepper seed that eventually also increased mortality on the pepper seed during week time. At the first week, the mortality percentage showed 6%, increased up to 9% on the later week and reached up to 76% on the seventh week. This number of mortality gives high losses towards farmers if they use unhealthy seed for a large plantation.

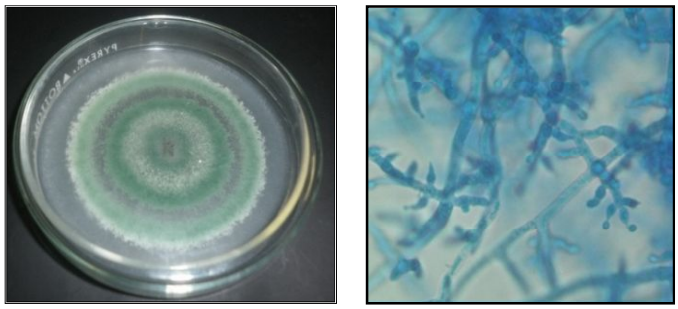
Phytophthora capsici isolate used for propagation and inoculation purposes in this experiment was obtained from Kendari, South East Sulawesi. The observation showed that fungus colony formed star-like pattern (stellate) with white color and thin. Microscopically, the sporangium formed oval shape with obvious papilla at the edge side, with length 31.3-36.4 m and width 22.8-25.9 m. The colony and sporangium of *P. capsici* are shown in Fig. 5 a and 5 b. *P. capsici* is characterized by un-partitioned and branched hyphae with papilla obviously showed in the tip of the sporangium. The colony patterns vary widely, ranging from smooth with no pattern up to thick with star-like pattern (*stellate*).



a b

Figure 5. The *P. capsici* fungi that causes pepper root rot (a). *P. capsici* colony in V-8 medium at seventh day; (b). *P. capsici* sporangium

Microscopic observation of *T. harzianum* obtained from isolation showed colony with hyaline color and changed to greenish white and light green particularly on the spot which had huge amount of conidia (Fig. 6a). The mycelium has septa hyphae and branches with diameter around 10.9-12.2 m. The main branch of conidiophores has diameter of 8.06-11.4 m and produces many side branches that can grow one-on-one but mostly formed in a loose group and develops into areas such as ring. The tip of conidiophore has 1-3 *phialids*, short with the both tips tapered. Compared to the middle sized with 16.3-18.1 m, the *phialid* looks slim and long especially at the apex of the branch as shown in Fig. 6b, the conidia at the tip of *phialid* is round-shaped, flat walls with dark green, whitish green, light green or with 6.97-12.3 m and the average size is 9.63 m.



a b

Figure 6. The isolate of Trichoderma harzianum (a) T. Harzianum colony at PDA medium, 5 days, (b) T. Harzianum *phialid*

In vitro antagonism test refers to dual culture method using V-8 medium. The observation of *T. harzianum* antagonism inhibition on *P.capsici* pathogen was conducted at the third day up to seventh day. The result is shown in Fig. 7. The result in percentage value is shown in Table 2.

Table 2. Percentage Value of T. harzianum Inhibitionon *P. capsici* During Antagonism test

Test	Inhibition percentage at the day(%)				
	3	4	5	6	7
1	10.71	11.43	17.50	20.00	23.08
2	25.00	31.43	32.50	36.67	44.23
3	17.85	25.71	27.50	31.11	42.31
4	28.57	31.42	32.50	35.56	40.36
5	32.14	34.28	37.50	40.00	46.15
6	30.36	32.88	36.25	38.89	47.12
7	32.14	37.14	4000	42.22	48.05
8	10.71	17.14	20.00	26.67	26.92
9	28.57	34.28	35.00	37.78	42.31
10	46.42	54.29	57.50	62.22	65.38
Average	26.26	3100	33.63	37,11	42.59

Table 2 shows *P.capsici* inhibitionby *T.harzianum* at the third days and the percentage value increased up to the seventh day of incubation. Based on the average of inhibitionpercentage, the lowest value was shown at the third day and perpetually increased in every observation

during the fourth up to the seventh day (Fig. 7). Inhibitionwas not shown during the first and second day of observation as both of fungi did not affect each other due to the distance of the two cultures of the fungi was wide enough i.e. 3 cm. But at the third day, the two cultures of fungi got close to each other indicated by forming inhibitionzone of *T. harzianum* against *P. capsici* as shown in Fig. 7c.

This inhibition zone did not have permanent size during observation as indicated in the seventh day of observation; the width of the zone became narrow. Besides, the growth of *T.harzianum* colony became faster with the diameter occupied V-8 petri dish which caused the size of *P.capsici* closing near to *T.harzianum* became smaller than *P.capsici* which keep away from *T.harzianum.* The diameter of *P. capsici* colony was smaller than the control treatment. This result found that the growth of *P.capsici* became distracted and running out of space to grow.

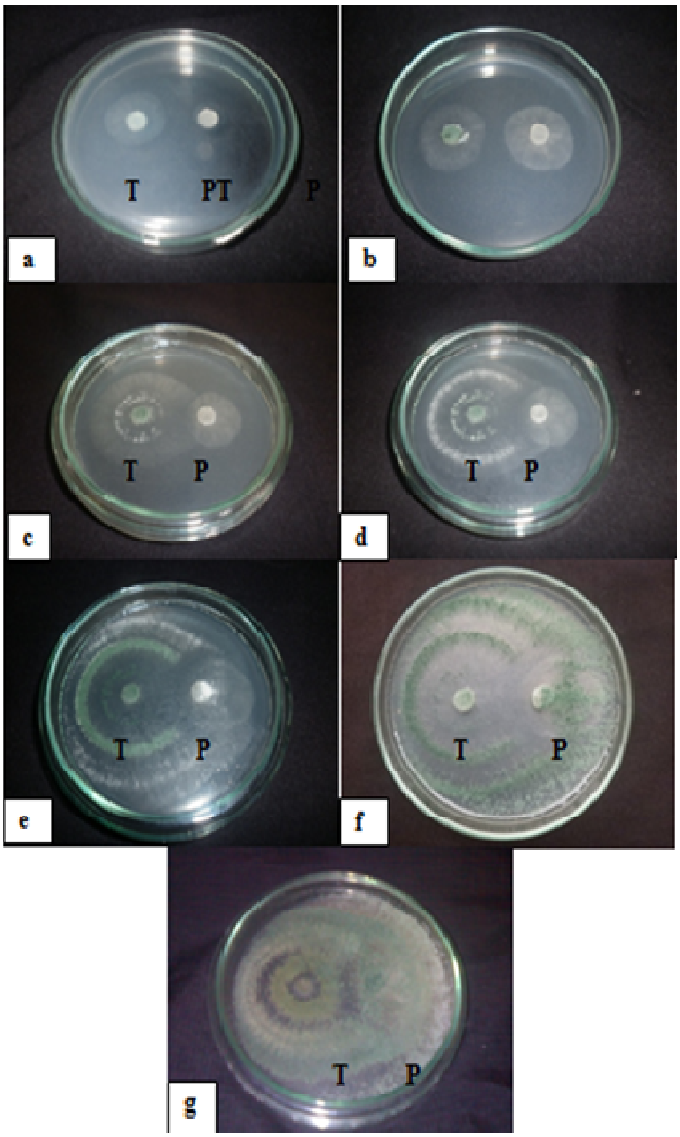


Figure 7. Inhibition test of Trichoderma harzianum against Phytophthora capsici on V-8 medium in a petri dish during (a) 1 day; (b) 2 days; (c) 3 days; (d) 4 days; (e) 5 days; (f) 6 days; and (g) 7 days

In antagonism test, the colony between *T. harzianum* and *P. capsici* experienced competition in possessing space and nutrients for living. This was indicated by rapid growing of *T. harzianum* and then inhibits the growth of *P. capsici.* Hawker (1950) *cit* Purwantisari *et al.* (2009) stated that competition in possessing space and nutrients for living between two interacted fungi can put the other fungus along its edge of the colony. Golfarb *et al.* (1989) *cit.* Purwantisari *et al.* (2009) conformed that fungus with rapid growth will occupy more living space and then inhibit the growth of other fungus.

Analysis of variance showed significant different among treatments towards disease percentage. The disease development was calculated using Van der Plank equation (1963) with different length time application of *T. harzianum.* This can be shown in infected plant and disease development of pepper root rot in Table 3.

Table 3. Percentage of Infected Plant and Disease Developmentof Pepper Root Rot

Treatment of *T. harzianum* application	Infected plant (%)	Disease development rate per unit per day
Control	100[a*)]	0.07
0 week before planting	100[a]	0.06
1 week before planting	100[a]	0.02
2 weeks before planting	93[b]	0.02
3 weeks before planting	80[c]	0.01
4 weeks before planting	80 [c]	0.00

Table 3 shows that the disease percentage is around 80 to 100%. The highest disease occurred at the plants without *T. harzianum* application (control treatment), 0 week before planting (S1) and 1 week before planting (S2) reached up to 100%. The lowest occurred at the plants with *T. harzianum* application at 4 weeks before planting (S4) which reached up to 80%. Those high numbers occurred at S0, S1, and S2 was caused by short length time of application which made the growth of *T. harzianum* population has not reached maximum condition. In addition, *T. harzianum* as cellulose decomposer has not provided sufficient nutrients which caused the plants experienced lack of nutrients and more susceptible to disease.

The highest disease development rate occurred at control treatment or without *T. harzianum* application which meant that there were no antagonist fungi inhibiting the development of disease. The lowest disease development rate occurred at *T. harzianum* application four weeks before planting (S4 treatment). Cook and Baker (1982) stated that the longer application time of *T. harzianum* on soil before planting could make the fungus to reproduce and adapt with the environment that lead higher potency for higher growth and colonize in inhibiting disease. Considering that the success of biological agent using *T. harzianum* is highly influenced on the length of antagonist interacted with pathogenic fungus suggest that early application treatment will have higher ability to reduce disease rate of root rot on pepper seed.

Based on the observation in the study site, the temperature and relative humidity were 22-28 °C and 79-100 % which appear as appropriate growth condition for pathogenic fungus. The growth of pathogenic fungus was also supported by higher rate of rainfall during observation. This condition produced high relative humidity of air and soil and also provided appropriate condition for pathogenic fungusto coverage their live space. These conditions caused increased of disease rate. In rainy season, low temperature and high relative humidity as well as sufficient nutrients will support spore germination of pathogenic fungus. The rain drop falling to the ground is believed to transmit the disease source in the form of propagule from the soil to the leave or un-infected plant. If the rain is accompanied by wind, the sporangium is released, fly and spread to the rest of pepper plant.

Microorganism can grow well in optimum environment temperature i.e. 24-28 °C. The optimum condition of soil temperature for fungus development and growth is ranges from 26-28 °C. In wet condition, pathogenic fungus is widely growth and the sporangium and zoospore will spread and infect the root plant. Increased population of pathogenic fungusin the soil will increase its potency to affect disease. The disease intensity of a plant highly depends on the number of existing pathogen (Prasad et al, 2002).

Analysis of variance showed that average value of plant height and number of leaves was significantly different among treatments. Application treatment of *T. harzianum* 0, 1, 2, 3 and four weeks before planting affected the plant growth indicated by the average value of plant height and number of leaves which shown in Table 4.

Table 4. The Effect of T. harzianum Application on Average Plant Height and Number of Leaves

Treatment of T. harzianum application	Average plant height (cm)	Average number of leaves
Control	24.40 a*)	2.00a*)
0 week before planting	27.16ab	2.33ab
1 week before planting	29.95b	3.50bc
2 weeks before planting	29.40b	3.00abc
3 weeks before planting	29.38b	4.25cd
4 weeks before planting	35.63c	5.33d

*Data with the same letter in the same column indicates un-significant difference based on Duncan test (DMRT) 5%

Table 4 shows that the highest average plant height occurred at S4 treatment i.e. 35.63 cm and the lowest was control treatment i.e. 24.40 cm. The highest average number of leaves also occurred at S4 treatment i.e. 5.33 and the lowest was 2.00. Length time application of *T.harzianum*4 weeks before planting affected higher height plant and number of leaves compared to control treatment. This indicates that S4 treatment have better performance to support the plant growth. The plant growth is affected by microorganism availability in the soil as decomposer and has antagonist ability against pathogenic fungus. *Trichoderma* sp., fungusis known as good organic decomposer in the soil which provides sufficient nutrients for plant and affects the plant growth. In addition, it is also recognized that this funguscan produce suppressive soil which provide good condition as plant medium. Besides of its antagonist mechanism through competition, antibiosis, hyper parasitism, and lytic enzyme producer, *T. harzianum* is known to have ability in stimulating plant growth.

Plant also needs nutrients for its development and growth. It can be seen in plant height chart, S4 treatment shows the highest average plant height which means that the nutrients is sufficient for the pepper plant growth. Fig. 8 and 9 show average value of plant height and number of leaves at each treatment.

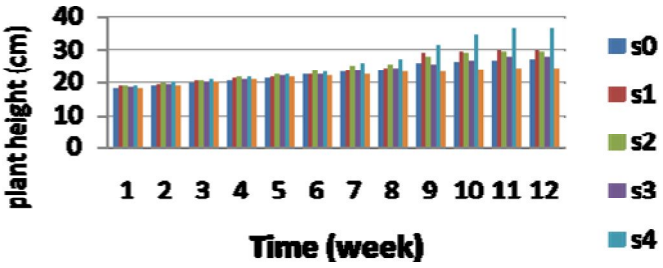


Figure 8. Average value of plant height at each treatment

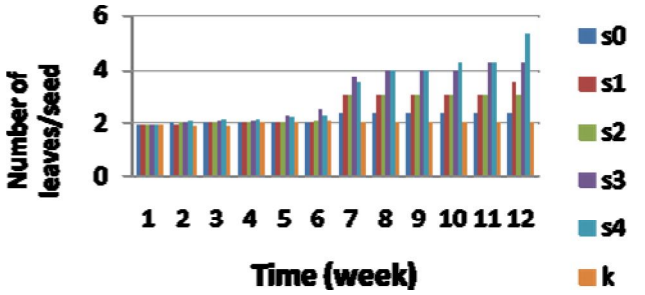


Figure 9. Average number of leaves at each treatment

Remarks: K: control, without *T.harzianum;* S0: application time of *T. harzianum* inoculum for 0 week before planting; S1: application time of *T. harzianum* inoculum for 1 week before planting; S2: application time of *T. harzianum* inoculum for 2 weeks before planting; S3: application time of *T. harzianum* inoculum for 3 weeks before planting; and S4: application time of *T. harzianum* inoculum for 4 weeks before planting.

IV. Conclusions

The application of *Trichoderma harzianum* is able to inhibit the root rot disease and the growth of pepper seed. In vitro antagonism test showed that *T. harzianum* is able to inhibit the growth and development of Phytophthora *capsici*. Length time application of *T. harzianum* 4 weeks before planting (S4) could produce the lowest percentage of infected plant and disease development rate among others treatments. This treatment could increase the growth of pepper seed including the height plant and number of leaves.

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