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The effect of media growing formulations on the effectivity of Endophytic Fungi isolated from roots to control fusarium wilt disease on Tomato

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Pathogen *Fusarium oxysporum* is a wilt disease that suppress the cultivation of tomato. The disease causes distress on the farmers because it has wide range of spectrums and can survive in the soil for years, despite the absence of a host. Synthetic pesticide method has so far been used to control the disease, but the method causes many problems and brings about contaminations for the environment and consumers. Environmentally friendly methods such as the use of endophyte fungi are needed to be applied to the level of farmers. The aim of the study was to evaluate the effectivity of endophytic fungi isolated from roots when combined with agricultural wastes to suppress wilt disease on tomato. The agricultural wastes namely paddy husk, sago waste, and cocopeat. The experiment was designed using factorial completely randomized design consisting of nine treatments and three replications. Field evaluation indicated that the formulation of cocopeat + 25% sticky rice had the best effects on the growth and survival of the plants. It resulted average height was 89.56 cm, stem diameter was 0.86 cm, and was able to suppress the incidence of fusarium wilt disease to 100% at vegetative phase.

Keywords: ***Endophytic fungi, Fusarium wilt disease, Tomato plants***

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is an ecenomically important crop and classified as a priority plant to be developed by research and development centre for Indonesian horticulture. So far, there has been an increase in tomato demand both from domestic and overseas. Data released from Horticultural Agency of Southest Sulawesi Province reveled that the production of tomato in 2008 reaches 22,200 T within an area of 613 Ha. Among factors contributing to a high demand on tomato are better income and society well-being, good nutritious needs, and an increase in raw material for pharmacy industry.

In tomato cultivation, constrains such as disease attack are significant factor for productivity. One of important diseases in tomato is fusarium wilt diseases caused by *Fusarium oxysporum*. The symptomps include the plant looking yellowish in the stalk and eventually become withered. The infection of *F. oxysporum* occurs begins from young roots and then spread to the whole parts of plant. Water transportation system will be impeded causing the plant become withered (Sharma et al. 2005).

The application of synthetic pesticide to deal with pathogen has been inevitable, although the price is high and gives detrimental effects on environment. Therefore, environmentally friendly and naturally sustainable alternative pesticide is needed. The use of biological agents as pathogen control is a biotechnological contribution to increase the productivity of cultivated plants (Thakuria et al. 2004). Due to biological and

environmental contamination from using synthetic pesticide, the application of biological agents is increasingly popular. The use of biological agents in an agricultural system have several benefits, such as protect plants during their life cycle, some types of microorganism are able to produce growth hormone (Silva et al. 2004; De Silva et al. 2018), undertake nitrogen fixation (Bai et al. 2003), and dissolve phosphate nutrient (Faccini et al. 2009) .

Endophyte fungi infects healthy plants in certain tissues and able to produce mycotoxins, enzymes and antibiotics (Carrol, 1988; Clay, 1988; Sun et al. 2005). Moreover, Owen and Hundley in Firakova et al. 2007 added endophyte microbes can synthesize chemical substances in plants. Endophytic fungus *Piriformospora indica* can increase tolerance of barley to high level of pH, enhance the production of antioxidants in roots, and induct systemic resistant (ISR) (Waller, et al., 2005). The endophytic fungus *Chaetomium globosum* can control clubroot diseases in broccoli (Asniah et al. 2013).

The aim of the study was to evaluate the effectivity of growing media formulations of endophyte fungi when combined with agricultural wastes to suppress fusarium wild diseases on tomato.

MATERIALS AND METHODS

Materials

Materials used during the study were endophytic fungi *Gliocladium* sp., and *Trichoderma* sp., isolated from roots of tomato. Meanwhile, the formulations were agricultural wastes (paddy husk, coconut fiber, and sago waste).

Experimental Design

The experiment was designed using factorial completely randomized design and conducted in nine treatments. The treatments were the combination of paddy husk + 0% sticky rice, paddy husk + 25% sticky rice, paddy husk + 50% sticky rice, sago waste + 0% sticky rise, sago waste + 25% sticky rice, sago waste + 50% sticky rice, cocopeat + 0% sticky rice, cocopeat + 25% sticky rice, and cocopeat + 50% sticky rice. Each treatment used three plants and repeated for three times. Overall, there were 81 experimental units.

Research Implementation

The research consisted of several steps started from the preparation of inoculum pathogen and two isolates endophytic fungi, multiplication of isolates, preparation of seed and planting media, the application of pathogen and biological agents, and observation.

Preparation of Inoculum Pathogen

Fresh pathogen *Fusarium oxysporum* was made and tested in *Potato Dextrose Agar* (PDA). Four pieces of seven days-old *F. oxysporum* colony in PDA media was sub-cultured in sterilized PDB media (200 mL), shake 150 rpm and incubated at 27^{o}C for 14 days until mycelium was formed. After 14 days, mycelium was harvested by filtering pathogen suspension using multi-layered sterile gauze. All the filtered suspension washed with sterile water and then filtered again and suspended with 500 mL of sterile water. The suspension of mycelium was mixed with sterile sand with a concentration of 1:5 (v/w) and used for inoculums source. Sterile sand containing pathogen mycelium crust were inoculated into planting media to 5 g per polybag in the treatments using pathogen then incubated for a week pro to be tested with endophytic fungi.

Media Formulation and inoculation of endophytic fungi

The media used during the study was the formulations of agricultural wastes such as paddy husk, sago waste and cocopeat. The cocopeat was cut into 0.5-1 cm. The agricultural wastes were immersed for 24 h, and then squeezed to reduce their water content to around 60%. Those wastes were then weighted to 250 g, put into thermal proof plastic bags and then sterilized using autoclave at 121^{o}C, 1 Atm for 15 minutes. Before mixed with formulation, the isolates were multiplied using PDA medium and incubated for 7 days prior to be mixed with agricultural wastes. After that, spore was harvested to obtain suspension by transferring 10 mL sterile distilled water to endophyte fungi and the surface of colony was scoured using spatula. The suspensions were then mixed with sterile formulation materials with the ratio of 1:25 (v/b). Biological agents in the formulation were incubated at room temperature for 6 MSI. The applications of endophytic fungi were conducted when they were transferred to the plants.

Soil, Planting and Seeds Preparation

Soil was obtained from the garden of Agricultural Faculty of Halu Oleo University. The soil was initially sterilized using steam to remove contaminations. Then it was mixed with sterilized manure (1:4 v/v) and put in 8 kg polybag to be used for planting media during the experiment. The tomato seeds used were the ones susceptible to Fusarium disease. The seeds were disinfected using 2% $NaOCl_2$ for 2 minutes and continued to be washed using sterile distilled water three times.

Average height, stem diameter, the incidence of diseases and plant production (crown and root weight) were measured every week for five times after the inoculation of pathogen. The incidence of diseases was calculated using the following formula:

KP = A/B x 100%

KP = diseases incidence

A = the number of suspected plants

B = the number of observed plants

Data were subjected to an analysis of variance (ANOVA) at α 0.05. Duncan Multiple Range Test (DMRT) was conducted to measure the specific differences between pairs of means.

RESULTS AND DISCUSSION

The results showed that agricultural wastes used for formulation materials significantly affected the growth of tomato and protect the plants from fusarium wilt disease. The average height, stem diameter, and disease incidence are presented in Table 1, 2, and 3, respectively.

Table 1 show that the treatment significantly affects the growth of tomato with the highest average height and stem diameter is obtained at the formulation of cocopeat + 25% sticky rice.

Table 2 show that treatment using cocopeat + 25% sticky rice significantly affects the growth of tomato with the highest diameter was 89.56 cm found in 4 MST.

There were no external signs of fusarium wilt disease seen in all treatments from the beginning to the third week of the observation time. The disease, however, was seen at the fourth week with the average incidence is presented in Table 3.

Tabel 1: The effect of the formulation materials on the average height of tomato in different observation times.

Treatment	Average height (cm) during the observation time…MST			
	1	2	3	4
Paddy husk + 0% sticky rice	19.29	30.93 ab	57.28 ab	87.30 ab
Paddy husk + 25% sticky rice	20.58	30.40 ab	51.72 ab	81.13 ab
Paddy husk + 50% sticky rice	19.64	30.37 ab	51.72 ab	83.94 ab
Sago waste + 0% sticky rice	18.69	24.68 b	44.28 b	74.83 ab
Sago waste + 25% sticky rice	18.14	26.33 b	46.28 b	72.44 b
Sago waste + 50% sticky rice	19.79	26.59 b	49.02 ab	78.56 ab
Cocopeat + 0% sticky rice	19.68	28.21 ab	56.13 ab	83.39 ab
Cocopeat + 25% sticky rice	21.51	33.63 a	62.08 a	89.56 a
Cocopeat + 50% sticky rice	19.61	29.40 ab	47.37 b	77.22 ab

Numbers followed by the same letter in each treatment indicate that there is no significant difference based on Duncan Multiple Range Test at α 0.05.

Table 2. The effect of formulation materials on the stem diameter of tomato in different observation times

Treatment	Average stem diameter (cm) during the observation time …… MST			
	1	2	3	4
Paddy husk + 0% sticky rice	0.35 ab	0.44 ab	0.61	0.80 ab
Paddy husk + 25% sticky rice	0.30 bc	0.33 c	0.57	0.73 b
Paddy husk + 50% sticky rice	0.28 bc	0.39 bc	0.59	0.85 a
Sago waste + 0% sticky rice	0.26 c	0.34 c	0.54	0.76 ab
Sago waste + 25% sticky rice	0.29 bc	0.33 bc	0.53	0.77 ab
Sago waste + 50% sticky rice	0.28 bc	0.37 c	0.58	0.72 b
Cocopeat + 0% sticky rice	0.35 ab	0.46 ab	0.59	0.81 ab
Cocopeat + 25% sticky rice	0.41 a	0.47 a	0.70	0.86 a
Cocopeat + 50% sticky rice	0.30 bc	0.36 bc	0.57	0.71 b

Numbers followed by the same letter in every treatment indicate that there is no significant difference based on Duncan Multiple Range Test at α 0.05

Table 3: The effect of formulation materials on the incidence of fusarium wilt disease (%) during vegetative phase in Tomato

Treatment	Average incidence of fusarium wilt disease (%) during the observation time…MST	
	3	4
Paddy husk + 0% sticky rice	0.00	22.22
Paddy husk + 25% sticky rice	0.00	0.00
Paddy husk + 50% sticky rice	0.00	22.22
Sago waste + 0% sticky rice	0.00	11.11
Sago waste + 25% sticky rice	0.00	0.00
Sago waste + 50% sticky rice	0.00	0.00
Cocopeat + 0% sticky rice	0.00	11.11
Cocopeat + 25% sticky rice	0.00	0.00
Cocopeat + 50% sticky rice	0.00	0.00

The highest incidence was observed in the formulation of paddy husk + 0% sticky rice and paddy husk + 50% sticky rice, while the treatment using cocopeat + 25% sticky rice was found free from diseases.

DISCUSSION

Endophytic fungi and their host plant perform mutualism relationship (Carroll 1988 & Clay 1988). Such relationship leads to the destruction of cells or tissues of plant, increase survival and photosynthesis rates of cells infected by pathogen. Moreover, the symbiotic relationship assists plants to be more tolerant toward biotic and non-biotic factors (Busby et al, 2016; Jones et al. 2016). The present study showed that the formulation of agricultural wastes mixed with sticky rice had better growth parameters, making the plans able to survive against *Fusarium oxysporum*.

Tomato treated with agricultural wastes and sticky rice produced better growth rates and

protection against fusarium wilt diseases when compared to other treatments. The combination of sticky rice with agricultural waste may provide required nutrients for the growth of endophytic fungi. Therefore, population of the fungi can infect the plants and then stimulate growth and protection against pathogen attack. Agrios (2005) reported that one factor affecting the ability of microbe to infect plants is the number microbial of population. A low incidence of diseases observed in the plants treated with *Gliocladium* sp., + *Penicillium* sp., could be caused by earlier colonization of the endophytic fungi in roots of the plant than pathogen plus the assistance of antibiotic mechanism. Endophytic fungi produce metabolites that affect physiological and biochemical of host (Clay, 1988). One metabolite produced by grass endophytic fungi is alkaloid, which is beneficial for protecting plants against herbivorous attack (Sellose, et al., 2004). The activity of organism can influence oil fertility so that it stimulates better growth for the plants. Setyowati et al. (2003) explained that the height and number of leaves lettuce fertilized by endophytic microbe is better than those which are not fertilized by the microbe.

The application of endophyte fungi combined with agricultural waste showed better growth rates and protection against pathogen attack as compared to single endophyte. The combination of endophyte fungi with agricultural waste should consider about agonistic mechanism done by every isolate so that the combination of isolate will be beneficial for the plants.

The combination of cocopeat + 25% sticky rice gave the best growth of all treatments and successfully protected the plants from pathogen. This could be due to the nutritional content required by endophyte fungi was higher in coco peat than in paddy husk and sago waste.

CONCLUSION

To sum up, the treatment using the combination of cocopeat + 25% sticky rice had the best effect on the growth and survival of the plants. It resulted average height was 89.56 cm, stem diameter was 0.86 cm, and was able to suppress the incidence of fusarium wilt disease to 100% in 4 MST observation.

Next studies on the best dosages formulation of endophytic fungi are important in order to obtain appropriate biotechnological technic in biological control against plant diseases.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example WEP designed and performleed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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