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INDONESIAN MINISTRY OF FOREIGN AFAIRS,
GOVERNMENT OF WEST NUSA TENGGARA
AND PT.NEWMONT NUSA TENGGARA

PREFACE

On behalf of the University of Mataram it is a pleasure and a great honor for me to welcome you to Lombok Island and to this international seminar on economic, culture and environment. Unram is one of the state universities in Indonesia which was founded in 1964. At the beginning of its establishment, it was only Law Faculty followed by Economic Faculty few years later. Now, Unram has eight faculties, i.e. law, economic, agriculture, animal husbandry, engineering, medical, and science faculties with about 1000 more lecturers and 450 supporting staffs and 17 000 students. Last month, October 2010, we celebrated our 48th anniversary. 48 years old if we make an analogy as the age of a human being is certainly a mature age. We realize that Unram is as it is now, because of supports from both government and stakeholders. To respond to this, Unram will continue to improve its maturity in all aspects. For instance, in recent years, our university has significantly improved the competency and qualification of its staff. This can be seen from the increase in number of our staff who gained their Ph.D from both domestic and overseas universities, including the increase in number of the professors. This is relevant as an approach to indicate progress. Now, we try to work even harder to reach our goal to be one of the World Class Universities. Because, we have huge potentials that need to be tackled professionally.

We have wide range of dry land area in both Sumbawa and Lombok island. In terms of facilities, we have standard laboratories to conduct collaborative research. So far, we have joint research with Nagoya University, Jichi Medical School in Japan, Arizona State University in United States, La Trobe University in Australia, Utrecht University in The Netherlands, SOAS in England. We expect to have more joint research in the future as this global world requires. This is also imperative that we need more colleagues to collaborate with in handling related issues for the betterment of the future life.

However, let us see what is going on in our region where malls, supermarkets, housing complex are built on very productive lands which sometimes are not based on comprehensive environmental studies. We expect that not in a very long time Unram would show its existence and scientific contribution on this global trend. This seminar is one of the efforts to show the world that we exist and ready to work together hand in hand to face the world's common concern. There is an urgent need of saving our planet from an even detrimental destruction. One of them is climate and environmental change, another is economic and cultural change.

In terms of economics, the discourse among Asean countries to unify their currency system is an interesting issue to be addressed proportionally. This seminar is expected to contribute some principle ideas and strategies on how Asean should tackle their internal problems to reach harmonious cooperation among its members. In relation to culture, there is a massive change in this global world. We are expected to refer to our local wisdoms as a filter of values which are destructive. Those issues are addressed proportionally in this seminar. Therefore, I expect that we gather in this place to contribute our best to tackle relevant issues based on our own discipline.

Last but not least, we need to give a special appreciation to the committee of this seminar because of their efforts this important event could be realized. Having said that, please excuse any inconvenience that we may cause. Finally, have a great seminar and please enjoy your stay in Lombok. Wassalamu'alaikum Wr Wb .Thank you

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ABSTRACT

The research aims were to know effect of application time of endophytic fungus *T. polysporum* isolate ENDO-04 and saprophyte *T. harzianum* isolate SAPRO-07 in increasing induced resistance of several vanili clones to Fusarium stem rot disease and their effects to growth of vanili plants. Experiment was conducted in plastic house of Agriculture Faculty of Mataram University by using Completely Randomized Design with factorial experiment consisted of two factors. Application time of *Trichoderma* fungi factor consisted of four levels, ie: without application of *Trichoderma* fungi (control), ENDO-04 fungus was given at the same time with SAPRO-07, ENDO-04 was given two weeks before SAPRO-07 isolate, and ENDO-04 was given four weeks before SAPRO-07 isolate. Vanili clones factor consisted of three levels, ie: Timbenuh NTB clone, Jurang Malang NTB clone, and Malang JATIM clone. The result shows that: application of *T. polysporum* ENDO-04 isolate given at the same time or two and four weeks before application of *T. harzianum* SAPRO-07 isolate was effective in controlling Fusarium stem rot disease and can increase induced resistance vanili plants to stem rot disease either on Timbenuh NTB clone, Jurang Malang NTB clone, and Malang Jatim clone. Application of *T. polysporum* isolate ENDO-04 fungus given at two and four weeks before application of *T. harzianum* isolate SAPRO-07 fungus was more trigger the vegetative growth (elongation of leaf shoots/spiral shaped) compare to when the two fungi were given at the same time.

Key words:vanili, Fusarium stem rot disease, endophyte, saprophyte, Trichoderma

INTRODUCTION

Nowadays, development area and vanili production (*Vanilla planifolia* Andrews) in Indonesia are in North Sumatera, Lampung, West Java, East Java, Bali, West Nusa Tenggara (NTB), North Sulawesi, Middle Sulawesi, and South Sulawesi. Vanili planting area in Indonesia is about 15.937 ha but it remains 50% and most of them are not productive. One of the major problems is because of infection of *Fusarium oxysporum* f. sp. *Vanillae* fungus which caused stem rot disease (Ruhnayat, 2004).

This fungus attacks all parts of vanili plant and infection starts from plant cuttings because of infestation of this fungus in the soil. According to Hadisutrisno (*in*

Redaksi Trubus, 2004), 7-32 % of plant cuttings are already contaminated although the mother plants show no symptom, and more than 80% of vanili plants in Indonesia are infected. On mature plants death rate is 50-100% and shortened production age from 10 times of harvest to twice and even no production at all (Hadisutrisno, 2005). Productivity of vanili plants are very low between 0,2-0,5 kg/dry pod per plant while its potential reach 1,0-1,5 kg/dry pod per plant (Ruhnayat, 2004).

Stem rot disease of vanili is still hard to control until now using many different ways because the pathogen has defence structure clamidospora withstand in the siol as a saprophyte between 3-4 years (Sukamto dan Tombe, 1995). contagious is trough infected plant cutting so that dissemination become fast and widespread (Hadisutrisno, 2005) and resistance vanili clone is not found yet (Ruhnayat, 2004). Based on that, control alternative is needed trough increase of induced resistance with treatment of endophytic fungus *T. polysporum* ENDO-04 isolate dan saprophytic fungus *T. harzianum* SAPRO-07 isolate (Sudantha dan Abadi, 2006 dan 2007).

Induced resistance is plant resistance to infection of pathogen because plant has been infected by another microorganisms before either by the same species or different species (Abadi, 2003). Endophytic fungus is fungus that lives in health plant tissues without causing symptoms or damage on host plants (Petrini dan Petrini, 1985 *dalam* Davis *et al*., 2003), and saprophytic fungus or antagonistic saprobe is fungus that lives on organic matter debris and has capability to suppress growth of soil-borne pathogen of fungus (Abadi, 2003).

Based on isolation of healthy vanili plant tissues in vanili plantation in NTB was found 19 isolates of endophytic fungus with antagonistic character to *F. oxysprorum* f. sp. *vanillae* fungus by *in-vitro* test. From 19 isolates of endophytic fungus found, there were two isolates suppress effectively growth of *F. oxysprorum* f. sp. *vanillae* fungus *in situ* and increase induced resistance to stem rot disease i.e *Trichoderma* sp. ENDO-04 Jurang Malang stem (*T. polysporum*). This endophytic fungus can also trigger vegetative growth of cutting and vanili plants Timbenuh clone besides this fungus grows well on coffea leaves debris, lamtoro leaves debris, candlenut leaves debris, and gamal leaves debris (Sudantha dan Abadi, 2006). On experiment of decompotition of coffea leaves debris, lamtoro leaves debris, candlenut leaves debris, and gamal leaves

debris shown that this fungus can increase decompotition process (Abadi dan Sudantha, 2007).

Antagonistic mechanism of endophytic fungus is to suppress pathogen development so that plants become resistance because of antibiosis. Petrini (1993) stated that endophytic fungus yield alkaloid and mycotoxin so it can be used to increase plant resistance to disease. According to Dahlam, Eichenseer dan Siegel (1991), and Brunner dan Petrini (1992), that endophytic fungus yield biological active compounds in vitro i.e. alkaloid, paxillin, lolitrems dan tetranone steroid. Photita (2003 *in* Lumyong *et al*., 2004), stated that antagonistic endophytic fungus has high activity in enzyme production that can be used to control pathogen. *Neotyphodium* sp. endophytic fungus yield β-1,6-glucanase enzyme that look like enzyme yielded by *Trichoderma harzianum* and *T. virens* fungi (Moy *et al*., 2002).

From 19 saprophytic fungi found in rizhosphere of healthy vanili plant, there were 12 isolates of *Trichoderma* spp. Fungus that were effective to suppress pathogen growth of *F. oxysporum* f. sp. *Vanillae* fungus in vitro, and 8 isolates were effective to control stem rot disease in situ (Sudantha dan Abadi, 2007). Two of these fungus can trigger flowering earlier on seedling of Timbenuh vanili clone. One of these isolates is *Trichoderma* sp. SAPRO-07 Jurang Malang vanili (*T. harzianum*) (Sudantha, 2007). Windham *et al*. (1986) reported that *T. Harzianum* fungus can increase seeds germination and plant growth. Tronsmo dan Dennis (1977 *in* Cook dan Baker, 1983) stated that spraying conidia of *T. viride* dan *T. Polysporum* fungi to protect strawberry plants from rot disease can trigger flowering earlier.

Antagonistic saprophyte fungus can suppress soil borne pathogen fungus in three mechanisms. For examples, *T. Viride* fungus has ability to live as mycoparasit that can penetrate mycelium and clamidospore of pathogen fungus so that it become lysis and cristalization, antibiotic production (gliotoxin and viridin) that can suppress growth of pathogen fungus, and has ability to grow faster as a result there is competition in space and nutrition with another fungi (Baker dan Cook, 1982).

From the former experiments above it can be concluded that both isolates of endophytic and saprophytic fungi *Trichoderma* spp. have a potential to develop as biofungicide, decomposer, and bioactivator of growth and flowering of vanili plants. In other words, it can minimize the use of synthetic fungicide and synthetic chemical

fertilizer so that the production cost can be reduced. Because of that, further research on application time of endophytic and saprophytic *Trichoderma* spp. on several vanili clones was conducted. The research aims were to know effect of application time of endophytic fungus *T. polysporum* isolate ENDO-04 and saprophyte *T. harzianum* isolate SAPRO-07 in increasing induced resistance of several vanili clones to Fusarium stem rot disease and their effects to growth of vanili plants.

RESEARCH METODOLOGY

The experiment has been done in plastic house of Agriculture Faculty of Mataram University. The experiment was designed using completely randomized design with factorial consisted of two factors i.e:

Factor application time *Trichoderma* spp. Fungus (T) consisted of four levels ie:

t0 = without isolate of *Trichoderma* spp. Fungus
t1 = ENDO-4 isolate given at the same time with SAPRO-07 isolate
t2 = ENDO-4 isolate given two weeks prior to SAPRO-07 isolate
t3 = ENDO-4 isolate given four weeks prior to SAPRO-07 isolate

Factor vanili clone (V) consisted of three levels ie:
v1 = NTB Timbenuh vanili clone
v2 = NTB Jurang Malang vanili clone
v3 = Jatim Malang vanili clone

Treatments were combination of Factor *Trichoderma* spp. Fungus and vanili clones and each treatment repeated three times so that there were 36 experiment units.

Vanili clone cuttings about 40 cm, which collected from spiral-shaped that have not been flowering before from vanili plants that have been fruited before, were used. Before planting on seedbed, cuttings were washed using water flow to remove phlegm and dirt.

Treatments were conducted as follows: treatment t1 with endophytic and saprophytic fungi *Trichoderma* spp. were given at the same time. Treatment t2 with endophytic isolate was given two weeks before saprophytic isolate. Treatment t3 with endophytic isolate was given four weeks before saprophytic isolate. Afterward, vanili cuttings were planted in polybags with slope 20-30 ^{o} to make vanili cuttings spreaded easily on stakes. Medium used for planting vanili cuttings was soil, sand, and sterile manure with ratio 1:1:1 (v/v/v) about 3 kg in polybag size 15 x 35 cm. Inoculation and

without inoculation of *F. oxysporum* f.sp. *vanillae* suspension (25 cc with conidia density 10^7/cc) were done One week after planting.

Variables observed:

1. Incubation period: observation was done daily until the first symptom appeared.
2. Length of stem rot: observed after 4 and 6 weeks after planting. Criteria for determining induced resistance level of vanili seedlings to stem rot disease is based on Sudantha's criteria of resistance reaction (2007).
3. Length of leaf shoot/spiral shaped: leaf shoots/spiral shaped.

Data were analysed by using analysis of variance with 95% truth level. Honesty significant difference test was used If there was any significant difference between treatment with 95% truth level.

RESULT AND DISCUSSION

Result of variance analysis showed that interaction between application time of *Trichoderma* and vanili clone factors were significant difference to incubation period of stem rot disease, length of stem rot, and length of leaf shoot/spiral shaped. Average of incubation period of stem rot disease, length of stem rot, and length of leaf shoot/spiral shaped were presented in Table 1, 2, and 3.

Table 1. Average of incubation period of stem rot disease due to application time of endophytic and saprophytic fungi *Trichoderma* spp.

No.	Application time of endophytic and saprophytic fungi *Trichoderma* spp.	Average of incubation period of stem rot disease (day)		
		Vanili clones		
		Timbenuh NTB	Jurang Malang NTB	Malang JATIM
1.	Control (without ENDO/SAPRO)	9,33	28,67	39,67
2.	ENDO-04 (*T. polysporum*) Given at the same time with SAPRO-07 (*T. harzianum*)	-	-	-
3.	ENDO-04 (*T. polysporum*) Given two weeks prior to SAPRO-07 (*T. harzianum*)	-	-	-
4.	ENDO-04 (*T. polysporum*) Given four weeks prior to SAPRO-07 (*T. harzianum*)	-	-	-

Note: - mean that no infection of stem rot disease occurred to vanili seedlings until 8 weeks
after pathogen inoculation

Tabel 2. Average of length of stem rot disease due to application time of endophytic and saprophytic fungi *Trichoderma* spp. On several vanili clones

No.	Application time of endophytic and saprophytic fungi *Trichoderma* spp.	Average of length of stem rot vanili stem (%)		
		Klon vanili		
		Timbenuh NTB	Jurang Malang NTB	Malang JATIM
1.	Control (without ENDO/SAPRO)	85,67 a *) B **)	44,67 b B	19,67 c B
2.	ENDO-04 (*T. polysporum*) Given at the same time with SAPRO-07 (*T. harzianum*)	0,00 a A	0,00 a A	0,00 a A
3.	ENDO-04 (*T. polysporum*) Given two weeks prior to SAPRO-07 (*T. harzianum*)	0,00 a A	0,00 a A	0,00 a A
4.	ENDO-04 (*T. polysporum*) Given four weeks prior to SAPRO-07 (*T. harzianum*)	0,00 a A	0,00 a A	0,00 a A
	BNJ0,05	4,10		

Note: data 0 mean that no infection of stem rot disease occurred to vanili seedlings until 8 weeks after pathogen inoculation (data were transformed to Arcsin $\sqrt{x + 0,5}$ on analysis of variance)

*) Number at each row followed by the same small letter was no significant difference at $p \leq 0,05$.

**) Number at each column followed by the same capital letter was no significant difference at $p \leq 0,05$.

Table 1 and 2 shown that application time of ENDO-4 (*T. polysporum*) fungus and SAPRO-07 (*T. harzianum*) given at the same time or time interval of two and four weeks caused no infection of stem rot disease to vanili seedlings either on Timbenuh NTB vanili clone, Jurang Malang NTB vanili clone, or Malang Jatim vanili clone, while all control plants were infected by stem rot disease with different incubation period and length of stem rot (Figure 1 and 2). In control plants i.e. Timbenuh NTB vanili clone, incubation period of stem rot disease 9,33 days in average with 85,67 % length of infection. Jurang Malang NTB vanili clone, incubation period of stem rot disease 28,67 days in average with 44,67 % length of infection. Malang Jawa Timur vanili clone,

incubation period of stem rot disease 39,67 days in average with 19,67 % length of infection.

It can be concluded from this experiment that application time of ENDO-4 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) can increase induced resistance of vanili clone to vanili stem rot disease. For example, at control treatment Timbenuh vanili clone had stem rot disease about 85,67 % (very susceptible), but after treated with ENDO-4 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) at the same time or time interval of two and four weeks no infection of stem rot disease found or became very resistant. Figure 1 shown that vanili plants either Timbenuh NTB clone, Jurang Malang NTB clone, and Malang JATIM clone increased their resistance to stem rot disease after treated with ENDO-4 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) spp.

Ability of ENDO-04 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) in controlling stem rot disease were in close relation with their ability in controlling *F. oxysporum* f. sp. *Vanillae* pathogen. Sudantha (2007) stated that *in-vitro* ENDO-04 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) suppressed growth of *F. oxysporum* f. sp. *vanillae* pathogen through several mechanisms namely competition, mycopharasitism, and antibiosis. Chet and Baker (1980 *in* Cook and Baker, 1983) stated that *T. harzianum* dan *T. hamatum* secrete ß-(1,3) glucanase and chitinase enzyme that cause eksolysis to host hyphae of pathogenic fungus *Rhizoctonia solani* and *Sclerotium rolfsii*. Further, they said that *T. hamatum* is also secrete cellulase enzyme so that increase its ability as mycoparasit to *Phytium* spp.

Success of using *Trichoderma* spp. as biological control agents of plant pathogen has been reported by prior researchers. Marshal (1982 *in* Cook and Baker, 1983) reported that inoculation of *T. harzianum* on bean seeds to control *Rhizoctonia solani* can reduce damping off disease up to 65 %. Harman *et al*. (1981 *in* Cook dan Baker, 1983) stated that treatment of lobak seeds with spores of *T. hamatum* can prevent infection to occur by *Phytium* spp. Rachmawati, Ambarwati and Martoredjo (1995) reported that inoculation of *T. viride* into culture medium can prevent vanili seedlings from stem rot disease.

Table 3. Average of length of leaf shoot/spiral shaped of vanili due to application time of endophytic and saprophytic *Trichoderma* spp. on several vanili clones

No.	Application time of endophytic and saprophytic fungi *Trichoderma* spp.	Average of length of leaf shoot/spiral shaped of vanili (cm)		
		Vanili clone		
		Timbenuh NTB	Jurang Malang NTB	Malang JATIM
1.	Control (without ENDO/SAPRO)	6,00 a*) A **)	17,00 b A	20,00 b A
2.	ENDO-04 (*T. polysporum*) Given at the same time with SAPRO-07 (*T. harzianum*)	51,00 a B	52,33 a B	53,00 a B
3.	ENDO-04 (*T. polysporum*) Given two weeks prior to SAPRO-07 (*T. harzianum*)	58,00 a C	60,67 a C	63,00 a C
4.	ENDO-04 (*T. polysporum*) Given four weeks prior to SAPRO-07 (*T. harzianum*)	60,00 a C	61,33 a C	64,67 a C
	BNJ 0,05	5,64		

*) Number at each row followed by the same small letter was no significant difference at $p \leq 0,05$.

**) Number at each column followed by the same capital letter was no significant difference at $p \leq 0,05$.

It can be seen on Table 3 that compared to control, application time of ENDO-4 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) given at the same time or time interval of two and four weeks is not only increase induced resistance of vanili seedlings to stem rot disease but also trigger vegetative growth of vanili seedlings i.e. elongation of leaf shoot/spiral shape either on Timbenuh vanili clone, Jurang Malang vanili clone or Malang vanili clone. Application of ENDO-4 (*T. polysporum*) at time interval of two and four weeks prior to SAPRO-07 (*T. harzianum*) gives better growth of leaf shoot than application of both fungi is given at the same time. Sudantha (2007) reported that application of *T. polysporum* isolate ENDO-04 can trigger elongation of leaf shoot/spiral shape, while application of *T. harzianum* isolate SAPRO-07 can trigger formation of flower shoot, but when the two fungi applied together its effect is more dominate to formation of leaf shoot/spiral shape.

The healthy Timbenuh clone due to treatment of endophytic and saprophytic *Trichoderma* spp.	The healthy Jurang Malang clone due to treatment of endophytic and saprophytic *Trichoderma* spp.	The healthy Malang clone due to treatment of endophytic and saprophytic *Trichoderma* spp.

Figure 1. The healthy Vanili seedling of Timbenuh clone, Jurang Malang clone, and Malang clone due to treatment of ENDO-04 (*T. polysporum*) and SAPRO-07 (*T. harzianum*)

Infected Timbenuh clone by stem rot disease on control treatment	Infected Jurang Malang clone by stem rot disease on control treatment	Infected Malang clone by stem rot disease on control treatment

Figure 2. The infected seedlings of Timbenuh clone, Jurang Malang clone, and Malang clone by stem rot disease on control treatmen (without endophytic and saprophytic *Trichoderma* spp.)

The ability of *T. polysporum* ENDO-04 and *T. harzianum* SAPRO-07 isolates that can trigger growth are due to exertion of chemical substances or growth regulator difused into vanili plant tissue. Windham *et al*. (1986) reported that *T. harzianum* can increase seeds germination and plant growth. Plant growth regulators are organic compound that synthesized in one part of plant and transported to another parts of plant, and at very low concentration can cause a physiologic response namely: to trigger growth of stem, leaf, root, flower, or fruit (Salisbury and Ross, 1995). For four kinds of

auxin i.e. gibberelin, cytokinin, absisic acid, and ethylene, ethilene is presumed as a hormone yielded by *Trichoderma* spp. that can trigger growth of vanili plants. Salisbury dan Ross (1995) stated that several fungi lived in the soil can produce ethylene. Etilen is assumed to be exert by those fungi to help seed germination, to control sprout growth, to slow down infection of soil borne pathogen, and to trigger flowers formation. For examples, use of ethylene on pineapple can trigger ethylene synthesis on plants so that triggered pineapple flowering. On seeding plants, all plant parts produce ethylene, either on roots, stems, leaf, and flower. Ethylene is a hormone that is easily to evaporate so that it moves easily from one organ to another plant organ. Ethylene effect in plant tissue is to increase enzyme synthesis depends on target tissue. When ethylene triggers falling leaf, sellulase and other cell wall degraded enzyme appeared on absicion layer. If cells wounded, phenylalanine ammonylyase appeared. This enzyme is important in formation of phenolic compound which play a role in wounds recovery. If a certain pathogenic fungi attacked cells, ethylene induce plant to form two kinds of enzyme that degrade cell wall of that fungi namely ß-(1,3) glucanase dan chitinase (Boller, 1988 *in* Salisbury and Ross, 1995).

CONCLUSION ANDSUGGESTION

Based on result and discussion, it can be conluded:

1. Application of *T. polysporum* ENDO-04 isolate given at the same time or two and four weeks prior to *T. harzianum* SAPRO-07 application is effective in controlling Fusarium stem rot disease and can increase induced resistance of Vanili plants to stem rot disease either on Timbenuh NTB clone, Jurang Malang NTB clone, and Malang JATIM clone.
2. Application of *T. polysporum* ENDO-04 isolate given at two and four weeks prior to *T. Harzianum* SAPRO-07 application is more trigger vegetative growth (elongation of leaf shoot/spiral shape) compare to application of the two fungi given at the same time.

Based on the result, it can be suggested:

1. To increase induced resistance of seedlings and vanili plants to stem rot disease, and to trigger growth of leaf shoot/spiral shape, it can be considered to use ENDO-04 (*T. polysporum*) and SAPRO-07 (*T. harzianum*) isolates.
2. It need to be done further research in the field especially in endemic areas of Fusarium stem rot disease by using ENDO-04 (*T. polysporum*) and SAPRO-07 (*T. harzianum*).
3. It need to be developed further about their potential as biofungicide so that it will benefit economically and ecologically because the technology is environmentally saved.

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