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STUDY ON APPLICATION OF ENDHOPYTIC ACTINOMYCETES AND MYCORHIZAE TO INDUCE RESISTANCE TOWARD *Rhizoctonia solani* AND GROWTH PROMOTION ACTIVITY

Kajian Pemanfaatan Endofitik Actinomycetes dan Mikoriza untuk Menginduksi Ketahanan Terhadap *Rhizoctonia solani* dan Promosi PertumbuhanTanaman.

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ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) and endophytic Actinomycetes are plant associated microorganisms which can colonize plant parts without harm the plant hosts. The colonization of plants by the microorganisms is known as a symbiotic mutualism that needed to be studied for their application as biological control agents and plant growth promoters. Therefore, this study was designed to determine the ability of endophytic Actinomycetes dan mycorrhizal fungi as biocontrol agents of *Damping off* disease caused by *Rhizoctonia solani* on maize plants. An isolate of endophyticactinomycetes showed strong inhibition against the pathogen on in vitro assay was used on *in planta*leveltest. In this study, both AMF and Actinomycetes isolate were used to inoculate the maize plants individually or in combination to examine the ability of those microorganisms to confer protection against pathogen. In addition, three isolates of Actinomycetes were tested for their ability to enhance the growth of the maize plants by coating the seeds with the isolates. *In planta*assay shows that the combination of mycorrhizal fungi and Actinomycetes was capable to inhibit the growth of the pathogen up to 70%. Meanwhile, the *in planta* growth promotion activity testing results show that there was an increase in growth of maize seeds coated with Actinomycetes isolates compare to the uncoated seeds. However, no significantly different was noted among the treatments.

Keywords : *Endophytic Actinomycetes, Arbuscular Mycorrhizal Fungi, Rhizoctonia solani*

ABSTRAK

Fungi Mikoriza Arbuskula (FMA) dan endofitik actinomycetes adalah mikroorganisme yang berasosiasi dan dapat hidup dalam jaringan tanaman tanpa mengakibatkan kerugian bagi tanaman inang. Kolonisasi jaringan tanaman oleh mikroorganisme tersebut merupakan simbiosis mutualisme yang perlu dikaji untuk pemanfaatan peningkatan produksi tanaman. Untuk itu, penelitian ini dirancang untuk menguji penggunaan endophytic actinomyctes dan FMA sebagai agensia hayati dan promoter pertumbuhan. Isolat yang secara *in vitro* memperlihatkan tingkat penghambatan yang kuat selanjutnya digunakan untuk pengujian secara *in planta*. Dalam pengujian

tahap ini, baik FMA dan actinomycetes diaplikasikan kepertanaman jagungs ecara individual dan secara kombinasi untuk mengetahui kemampuan kedua jenis mikroorganisme melindungi tanaman dari serangan patogen. Selainitu, tiga isolate actinomycetes lainnya diaplikasikan kepertanaman jagung untuk mengetahui kemampuan isolate dalam meningkatkan laju pertumbuhan tanaman. Hasil pengujian *in planta* menunjukkan bahwa kombinasi FAM dan actinomycetes mampu menekan tingkat serangan pathogen sampai 70%. Hasil uji aktivitas pertumbuhan memperlihat kan peningkatan pertumbuhan tanaman jagung dengan aplikasi actinomycetes melalui seed coating, walaupun peningkatan laju pertumbuhan tersebut secara statistic tidak berbeda nyata dengan laju pertumbuhan tanaman tanpa pemberian actinomycetes.

Kata kunci : *Endofitik actinomycetes, Arbuscula Mycorhizae Fungi, Rhizoctonia solani*

INTRODUCTION

Maize (*Zea mays* L.) is the most widely grown cereal crop and has the highest production of all the cereals. It is an important food staple in many countries, as well as being used in animal feed and many industrial applications. With an increasing demand of this important plant worldwide, there is a need to increase the productivity to fulfill the market demand. The province of South Sulawesi as the central of maize national development area, has implemented the surplus maize production program since 2009 through valorization of maizeplantas a source of food and renewable energy. This Program was design in order to keep the availability of food for national prosperity and security.

In order to achieve the goal of the above program, besides an effort to increse the production of maize plant, identification of the certain factors that inhibit the optimal production must be determined.A main problem causing a significantly production declining is pathogens attacking.One of the important disease of maize plant is *damping off*disease caused by the fungus *Rhizoctonia solani*. The disease causes growth failure on almost all maize field growns. This pathogen is difficult to control through plant rotation and fungisida because its ability to survive as sclerotia under adverse environment condition for many years in soils and its activity as saprophytic on wide plant host range. Inspite of that, the application of chemical pesticides has bad impact on environmental, human being and other organisms. Therefore, an alternative strategy to control the

pathogen is necessary and become a topic of agri-cultural study in the last decades.

To date, plant associated bacteria and fungi are reliable alternative as the symbiositic of microorganisms with plants increase an abilty of plants to confer protection against pathogens through secrection of secondary metabolites by micro-organisms or through inhibitory plants producing enzymes that mediated by symbiotic microorganisms. These conditions occur when actinomycete bacteria reside inside plant tissues (Aghighi *et al.*2004; Coombs and franco 2003; El-Tarabily 2003; Cao *et al.* 2005) and also on symbiotic between plants and mycorrhizal fungi (Bertham, 2006; Hasbi, 2005; Kasiamdari, 2000). Several studies also have shown that interaction between plants and non-pathogen microorganisms can enhance plant growth and productivity (Rusdi *et al*. 2000; Tokala *et al.* 2002; Demir 2004; Trisilawati & Firman 2004).

In order to find an alternative for agrochemical, actinomycetes and mychorryza are two potential micro-organisms that can be used as biocontrol and growth promoter agents. In the present study, endophytic actinomyctes and FMA were used individually or in combinations to protect maize plant from soil borne pathogen. The plant growth promotion activity of theisolate actinomycete was assayed in planta to examine the ability of endophytic actinomycetes in enhancing growth of maize plants.

MATERIALS AND METHODS

In Planta Antagonism Assay

An isolate actinomycete that had a strong *in vitro* activity against *R. solani* (previous study) was used in a glasshouse experiment in combination with an arbuscularmycorrhizae fungus (*Glomus* sp.)to test the ability of the plant symbiotic microorganisms in protecting maize seeds against the soil borne pathogen. Inoculum actinomycete was prepared by growing the isolate on potato dextrose agar (PDA) medium and incubated at 27^{o}C for 2 weeks. The actively growing of colony containing spores of isolate was used to coat 100 g of maize seeds and planted into non-sterile sandy soil infected with *R. solani*. Control seedlings were treated in parallel with water without isolate actinomycete spores and planted into the same soil as

the positive control. Controls with no fungal inoculation were also run, in which untreated maize seeds were planted into uninfected soil. Every treatment was run in fiver eplicates with 5 plants in each replicate pot. Three days after planting, the soil was inoculated with FMA to allow the actively growing seedlings be infected by the FMA. A week after planting, the soil was infected with the pathogen.Pathogen inoculum was prepared by growing the pathogen in PDA for 2 weeks. 100 ml of sterile RO water was added to a plate covered with actively growing mycelia and spores of the pathogen. The plates were then scraped and 50 ml of the pathogen mycelia suspension was used to infect each pot filled with two kg of sandy soil.

Five treatments were used as follows:

1. MAR = *Mycorrhizae* + *actinomycete* + *R. solani*
2. MR = *Mycorrhizae* + *R. solani*
3. AR = Actinomycetes + *R. Solani*
4. K1 = Without *Mycorrhizae* + actinomycetes + *R. Solani* inoculums (Negative Control)
5. K_2 = Inoculum of *R. solani* (Positive Control)

In Planta growth promotion assay

The plant growth regulatory activity of the isolates was run using three selected isolate actinomycetes. Maize seeds were coated with the isolates as described above and sown in pots containing two kg of sandy soil and the pots were arranged randomly. The activity of the isolates to promote growth of the plants was assayed by measuring the height of the plants, number of leaves at 8 weeks after planting, andseedling emergence time was observed a day after planting. Plant growth promotion in this assay was expressed as a significantly increase of plants height, number of leaves and decreased in germination time of the treated plants compared to non treated plants. Data were analyze dusing Analysis variance at significantly different $p<0.05$.

RESULT AND DISCUSSION

Antagonism Assay

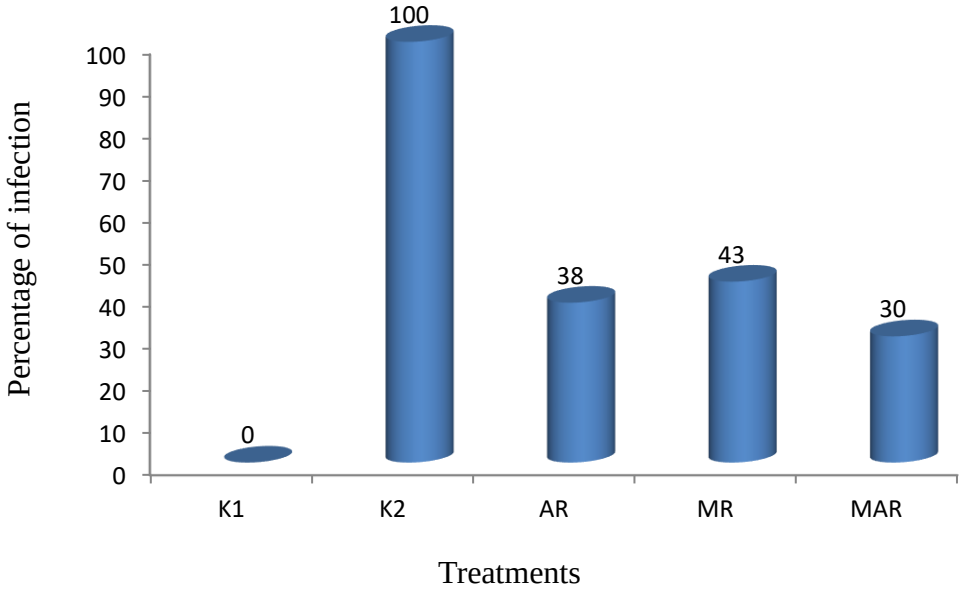
An antagonisme assay was done to determine the eficacy of the isolate actinomycetes andmycorryzal fungus in decreasing *R. Solani* infection rate on maize plant. The percentage of the patogen infection rate as shown in Picture 1.

The result giving in Figure 1 shows that both actinomycete and Glomus sp. were capable in protecting maize plants against *R.Solani*. A combination of actinomycete and FMA however performed better protection compared with single application of actinomycete or *Glomus* sp. The microorganism inoculums significantly reduce ($P<0.05$) disease incidence of the pathogen by at least 60%.

Actinomycete bacteria and AMF have been widely studied for their capacity to enhance plant growth, produceantibiotics, parasitize fungi and compete with deleterious plant pathogens. Several mechanisms have been also purposed to explain the capability of plant associated microorganisms in protecting plant against pathogens. Actinomycetes have mechanisms in controlling the growth of fungus pathogen such as *R. solani*through antibiotics and chitinase like enzymes production (Trejo-Estrada *et al*. 1998).Widyastuti (2003) explained that wall cell of fungi contain glucan and chitin, according to Amaranthus(2001), chitinase is an enzyme that hydrolyses chitin and utilize it as Carbon and Nitrogen sources. Hence, actinomycetes can protect plants from phytopathogenic fungi through productionof anti fungal like compounds.Tokala et al. (2002) claimed that *Streptomyces* spp. secrete hidroxamate- siderophores which inhibit the growth of Phytopathogen through a mechanism of iron competition and further Sessitschet al. (2004) suggested that competition and induced resistance which act individually or in combination to fight off pathogens.

The current finding of suppression of damping off disease incidence by Mycorryzalfungi when it was applied individually is similar to those found by Kareem and Hassan (2014) and on tomato and Hafez et al. (2013) on bean plants. Therefore, AMF areconsidered as bio-control agents. The protective impact of mycorryzal fungi against root pathogenic fungi is the result and output of the complex interactions between pathogens, plant and AMF (Harrier and Watson 2004).

Thus, several mode of actions by which AMF could protect plants from soil borne pathogen have been recognized. A symbiotic between plant and AMF resulted in improve-



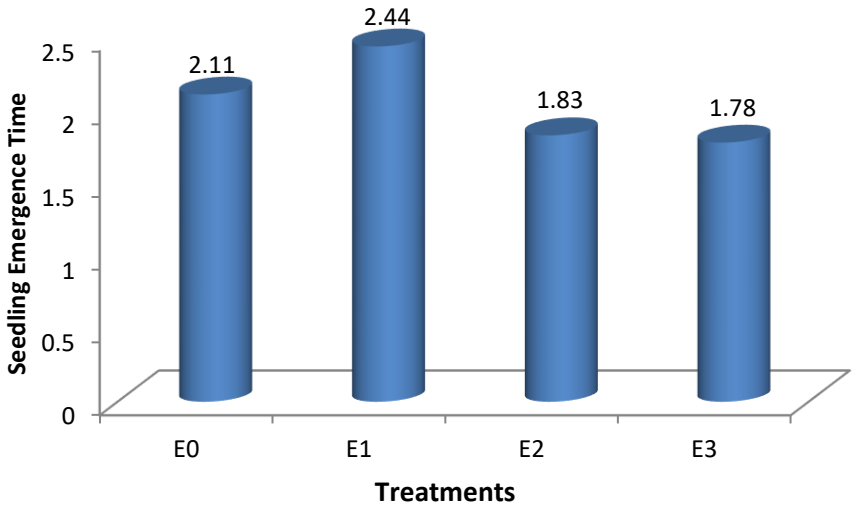
Picture1. Percentageof *R.* Solani infection rate on Maize plant in the present of antagonistic microorganisms

ments of plant growth followed by more root colonization by AMF that occurs as a result of enhancement of the mineral nutrient status of plants(Tahat et al. 2010). Enhancing in nutrient uptake resulted in more vigorous and healthy plants, which further act as a natural plant self defense against pathogens. Colonization of plant roots by AMF thick-walled hyphae may act also as physically barrier that could protect the roots from infection. Another mechanism of AMF in protecting host plant from pathogen is through production of bioactive compounds such as lignin, phenol andPhytoalexin (Harrier and Watson 2004; Kareem and Hassan 2014; Hafez et al. 2013).

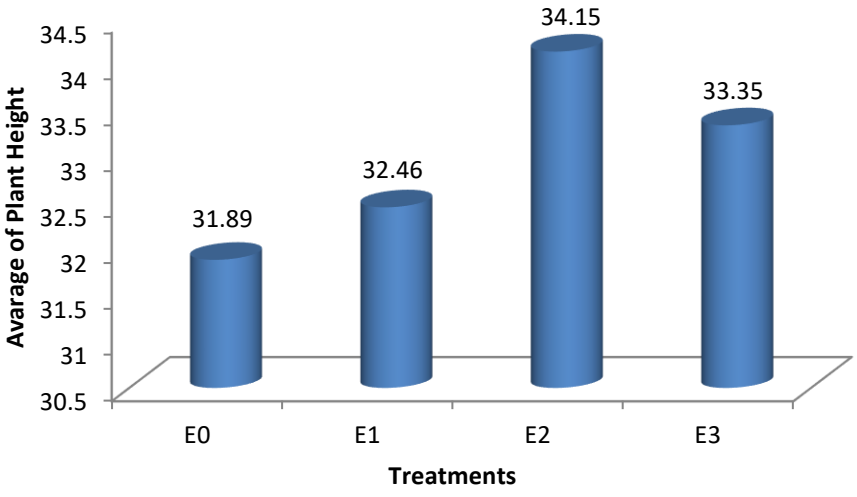
Application antagonist bacteri-um in combination with fungus showed better disease suppression than single application (Table 1).This better result was due to a combination and interaction of several mechanisms that played by the antagonists in protecting and increasing maize plant resistance against pathogen. This result is in accordance with a study by Sastrahida-yat et al. (2011) which found that a combination of AMF an infection dactinomycetes increased the resistance soybean plants against *Sclerotium rolfsi* Sacc.

Inplanta Growth Promotion Assay

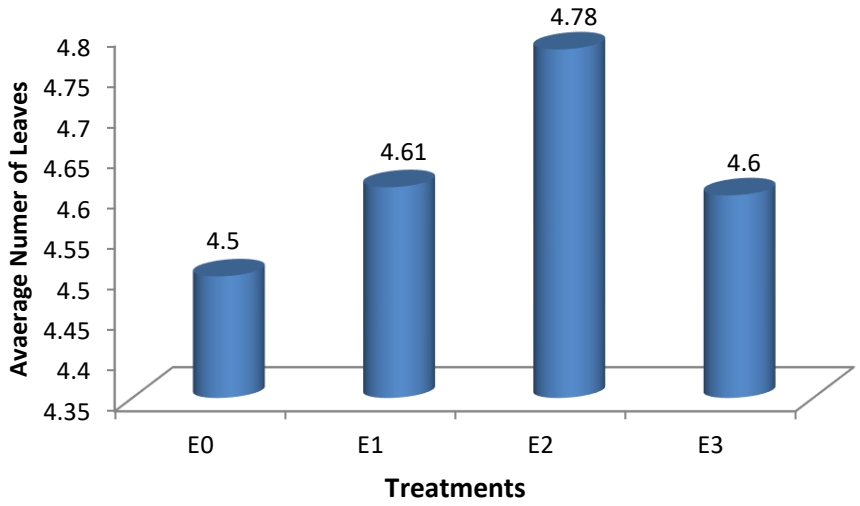
The ability of isolate actinomycetes to promote growth of maize plant was conducted *in planta*. The results show in figure 2 and 3 indicate that the isolate actinomycetes were able to promote growth in maize plants. Activity of the isolatesin promoting growth of maize plant was indicated by increased ofplants height, number of leaves and reduced on germination time (Picture 2, 3, and 4).



Picture 2. Seedlings emergence day of actinomycetes inoculated and non-inoculated maize plants



Picture 3. Stalk height of actinomycetes inoculated and non-inoculated maize plants



Picture 4. Number of leaves of actinomycetes inoculated and non-inoculated maize plants

The ability of isolates to promote growth of plant was conducted in planta. Three isolate actinomycetes (E1, E2, dan E3) were able to increase maize growth, but not significantly different from untreated plants. Isolates were able to increase plant height, number of leaves and improved time of emergence seedling of maize plants, but the results are not significantly different ($p>0.05$) as compared to the control. The mechanism by which plant growth is improved by the isolates may be similar to those exhibited by rhizosphere microorganisms and include the production of phytohormones. This is supported by the observation that plant growth regulators such as Indole Acetic Acids (IAA), cytokinin and gibberellin are also produced by rhizobacteria and actinomycetes which live in association with plants (Patten & Glick 1995; Doumbouet al. 2002; Sessitschet al. 2004). The plant growth hormones like IAA (auxin) and cytokinins are able to stimulate both rapid and long term responses in plants.

CONCLUSSION

AMF (Glomus sp.) and endofitik actinomyecetes are potential biological control agents which can confer protection against R. solaniindividully or in combination of them. Three isolates actinomycetes improved maize growth activities on in planta testing.

REFERENCES

Aghighi, S., Bonjar, G.H.S., Rawashdeh, R., Batayneh, S. and Saadoun, I., 2004, First report of anti fungal spectra of activity of Iranian actinomycetes strains against *Alternaria solani, Alternata alternate, Fusarium solani, Phytophthora megasperma, Verticillium dahliae* and *Saccharomyces cerevisiae*, Asian Journal of Plant Science, 3: 463-471.

Bertham Y.H. 2006. Interaksi Mikoriza Arbuskula dengan Tanaman Pangan dan Hortikultura dalam Modul Workshop Teknologi Baru Bekerja dengan Mikoriza. Kerjasama Asosiasi Mikoriza Indonesia dengan Pusat Penelitian dan Sumber Daya Hayati Bioteknologi IPB, Bogor.

Cao, L. Qiu, Z., You, J., Tan, H. dan Zhou, S. 2004. Isolation and characterization of endophytic Streptomyces strains from surface sterilize tomato (*Lycopersicon esculentum*) roots.Letters in Applied Microbiology, 39: 425-430.

Coombs, J.T. and Franco, C.M.M., 2003, Isolation and identification of Actinobacteria from surface-sterilized wheat roots, Applied and Environmental Microbiology, 69: 5603-5608.

Doumbou, C.L, Salove, M.K.H., Crawford, D.L. and Beaulieu, C., 2002, 'Actinomycetes, promosing tools to control plant diseases and to promote plant growth', Phytoprotection, 82: 85-102

El-Tarabily, K.A., 2003, 'An endophyticchitinase-producing isolate of *Actinoplanesmis souriensis*, with potential for biological control of root rot of lupin caused by *Plectosporium tabacinum*, Australian Journal of Botani 51: 257-266.

Hafez, EE.,Gamal, M., Fattah, A., El-Haddad, SA., Rashad, YM. 2013. Molecular defense response of mycorrhizal bean plants infected with *Rhizoctonia solani*, Ann. Microbiol. 63: 1195-1203.

Harrier, LA.& Watson, CA. 2004. The potential role of Arbuscular Mycorrhizal fungi in the bioprotection of plants against soil-borne pathogens in organic and or other sustainable farming systems. Pest Manage. Sci. 60: 149-157.

Harmet, T. Habazar, E.F. Husin, dan D.P. Putra, 2000, Peranan Cendawan Mikoriza Arbuskula dan Pupuk Fosfor dalam Meningkatkan Ketahanan Tanaman Kedelai Terhadap Penyakit Pustul Bakteri, Prosiding Seminar Nasional Mikoriza I.

Hasbi, R. 2005, Studi Diversitas Cendawan Mikoriza Arbuskula (CMA) pada Berbagai Tanaman Budidaya di Lahan Gambut Pontianak, Jurnal Agrosains 2.

Kareem, T. & Hassan, MS. 2014. Evaluation of *Glomus mosseae*

as Biocontrol agents against *Rhizoctonia solani* on Tomato. Journal of Biology, Agriculture and Healthcare, 4 (2): 2224-3208.

Kasiamdari, R.S., S.E. Smith, F.A. Smith &E.S. Scott, 2000. Effect of phosphorus on theinteraction between *Glomus* sp. and binucleate *Rhizoctonia* sp. or *Rhizoctonia solani*.Prosiding Seminar Nasional Mikoriza I, Indonesia.

Pattern, C.L. and Glick, B.R., 1995. Bacterial biosynthesis of indole-3-acetic acid', Canadian Journal Microbiology, 42: 207-220.

Rusdi, N., M.D. Suryadi & A.E. Tjahjono, 2000. Aplikasi pupuk hayati cendawan mikoriza arbuskula dan pupuk P pada budidaya tanaman ubi kayu (*Manihot esculenta* Crantz), Prosiding Seminar Nasional Mikoriza I, Indonesia.

Sastrahidayat IR., Djauhari S., Prasetya B., Saleh N. 2011. Biocontrol of damping-off disease (*Sclerotium rolfsii* Sacc.) Using Actinomy-cetes and Vam fungi on soybean and impact to crop production and microorganism diversity in rhizosfer zone.International Journal ofAcademic Research, 3.No. 6.

Semra, D., 2004, Influence of Arbuscular Mycorrhiza on Some Physiological Growth Parameters of Pepper, Turk J Biol, 28: 85-90.

Sessitsch, A., Reiter, B. and Berg, G., 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities', Canadian Journal of Microbiology, 50: 239-249.

Tahat, MM. Kamaruzaman, Sijam, Othman, R. 2010. Mycorrhizal Fungi as a Biocontrol Agent. Plant Pathology Journal 9 (4): 198-207.

Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H.,Deobald, L.A., Bailey, J.F. and Morra, M.J., 2002,'Novel plant microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*)', Applied and Environmental Microbiology, 68: 2161–2171.

Trejo-Estrada, S.R., Rivas, S.I. and Crawford, D.L., 1998, 'In vitro and in vivo antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turf grass', World Journal of Microbiology and Biotechnology, 14, 865-827.

Trisilawati, O. danFirman, C. 2004, Pengaruh Cendawan Mikoriza Arbuskula terhadap Pertumbuhan Bibit Panili (*Vanilla planifolia* Andrews). Buletin TRO, 15: 19-24.

Widyastuti S. M. 2003. Biological Control of (*Sclerotium rolfsii*) Damping Off of Tropical Pine (*Pinus merkusii*) with Three Isolates of *Trichoderma* spp. Journal Biological Science, 3: 95-102.