INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 1, p. 270-278, 2017

RESEARCH PAPER OPEN ACCESS

Isolation, identification and ability of endophytic fungi in stimulating cocoa seed germination

Muh Assad[1], Muhammad Taufik*[2], H. S. Gusnawaty[2], Asniah[2]

[1]*Assessment Institute for Agricultural Technology, Kendari, Indonesia*

[2]*Department of Agrotechnology, University of Halu Oleo, Kendari, Indonesia*

Key words: Cocoa, VSD disease, Endophytic fungus, *Paecilomyces* sp.

http://dx.doi.org/10.12692/ijb/10.1.270-278 Article published on January 29, 2017

Abstract

Cocoa is an important commodity of the world, with demands increasing every year. Indonesia is the third largest cocoa producer in the world and continues to increase its production. One of the factors limiting the production of cocoa is an infectious disease Vascular Streak Dieback (VSD), caused by *Ceratobasidium theobromae*. VSD disease can cause yield losses up to 50%, and severe infections cause the death of cocoa plants. This research was conducted using a survey method, isolation and identification of endophytic fungi based on morphological characters that were based on fungi identification book, as well as testing the ability of the fungi to stimulate germination of cacao seeds *in vitro*. The study was able to isolate and identify six isolates of endophytic fungi, namely *Paecilomyces sp* isolates EP1, EP3, EP6, and EP8, *Cladosporium* sp. and *Nigrospora* sp. *Paecilomyces* sp. isolate EP1 was endophytic fungus which had the ability to stimulate the germination of cacao seeds by 100%, followed by *Nigrospora sp*. (90%). Presumably, the endophytic fungi produced hormones to stimulate the process of cacao seed germination. One isolate of *Aspergillus* sp. can not be categorized as an endophytic fungus of cocoa plant. Endophytic fungus *Paecilomyces sp* isolate EP1 is promising to be used as an alternative environmentally friendly VSD disease control agent.

* **Corresponding Author:** Muhammad Taufik ✉ taufik24@yahoo.com

Introduction

Cocoa is an agriculture commodity which has the international market, so that cocoa-producing countries seek to increase cocoa production to meet the needs of the world. Indonesia is currently the third largest exporter of cocoa in the world, suppliying about 13% of world cocoa production (Binam *et al.*, 2008; ICCO, 2013; Ibiremo *et al.*, 2014). Indonesia national cocoa productivity is still relatively low (854 kg/ha) but can potentially be improved (Southeast Sulawesi Agriculture Department, 2013). Malaysian cocoa productivity reached 1,500 – 1,800 kg/ha (Azhar and Lee, 2004; Dormon *et al.*, 2004), while Papua New Guinea cocoa productivity was 1,200 kg/ha (CABI, 2016).

Cocoa productivity in Southeast Sulawesi, Indonesia was declined from 869 kg/ha in 2014 to 733.8 kg/ha in 2015 (Disbun Sultra, 2015). Extensive cocoa plantations in Southeast Sulawesi are dominated by smallholders, covering an area of 255,350 ha, with cocoa production in 2014 amounted to 161,514 tons (Disbun Sultra, 2015), and in 2015 decreased to 133,708 tons.

The decreases in cocoa production and productivity in Southeast Sulawesi had an impact on national cocoa production and productivity. This was due to the Southeast Sulawesi's cocoa cropping area was 251,730 ha, ranked second after Central Sulawesi's of 284,125 ha (Director General of Plantation, 2014). Southeast Sulawesi has been designated as a national cocoa region through the decision of the Minister of Agriculture No: 46/Kpts/PD.3000/1/2015. Many factors causing low production of cocoa, among others was vascular streak dieback (VSD) disease of *Ceratobasidium theobromae* (Rosmana *et al.*, 2010; Samuel *et al.*, 2011). VSD disease can cause yield losses by 40% - 50%, and even can be deadly to productive cocoa crops (Guest and Keane, 2007; Halimah and Sri-Sukamto, 2007; Rosmana *et al.*, 2010; Samuel *et al.*, 2012; Rosmana *et al.*, 2014).

Mc Mohan *et al.* (2010) reported that VSD has infected cocoa plant since 1980, in the District Ladongi, Ease Kolaka, Indonesia. Similarly, it has also been found in 1989 in Kolaka (Askindo, 2008).

Reports from the Department of Crops and Horticulture in Southeast Sulawesi (2013) noted the total VSD infected area of 634.8 ha. Taufik *et al.* (2015) reported that the prevalence of VSD in the centers of cocoa cropping areas in South Konawe (50.42%), Konawe (44.58%), Ease Kolaka (32.72%), North Kolaka (35.81%) and Muna (49.10 %).

Given the level of yield losses incurred by the VSD disease is potentially high, it is necessary to control the disease infections, one of them is by using endophytic fungi. Endophytic fungus is a fungus that lives and infect plant tissue but do not cause disease symptoms (Clay, 1992), but has activities against pathogen infection in plant tissues.

Hanada *et al.* (2010) and Daeley *et al.* (2009) reported some endophytic fungi on cocoa crops, namely six isolates of the genus Trichoderm, Pestalotiopsis, Curvularia, Tolypocladium and Fusarium which have activities against pathogen infection. Wahab *et al.* (2016) reported that the endophytic fungus *Trichoderma asperellum* can be combined with other biological agents to control the VSD on cocoa crops. Amin *et al.* (2014) found six genera of fungi endofiit *Curvularia* sp., *Fusarium* sp., *Colletotrichum* sp., *Geotrichum* sp., *Aspergillus* sp., and *Gliocladium* sp. which could potentially be used as biological control to control the VSD disease. VSD control with endophytic fungi is a new strategy to inhibit the growth of pathogenic VSD. Colonization in healthy plant tissues or the ability to produce mycotoxins, enzymes and antibiotics are ways to fight infectious pathogens in plant tissues (Simarmata and Rumilla, 2007). Therefore, the purpose of the research was to isolate, identify and test the ability of endophytic fungi to stimulate cocoa seed germination *in vitro*.

Materials and methods

Sample collection

Sampling was carried out at the Experimental Field, Sub Cocoa Station, Plantation Office of Southeast Sulawesi Province, at District Konda, South Konawe. Cocoa tree selected was cocoa tree that was VSD asymptomatic and VSD symptomatic,

with an attack rate of > 40% but still productive. Healthy leaf and petiole samples were put into plastic sample bags to be taken to Laboratoium.

Isolation of endophytic fungi

Isolation of endophytic fungi was conducted following the procedure of Asniah *et al.* (2014). Leaf and petiole samples were washed using running water for ± 5 minutes, then cut into pieces with a size of ± 1-2 cm. Surface sterilization was done by inserting pieces of leaf and petiole, in sequence, into a 70% alcohol for 1 minute, NaOCl solution for 1 minute, then rinsed with sterile distilled water three times, each for 1 minute.

The samples were then dried on sterile filter paper. The pieces of leaf and petiole were finally grown in three-point method on potato dextrose agar medium (PDA) and incubated for 3-7 days.

Stimulation test for cocoa seed germination and identification of endophytic fungi

The stimulation test was performed by growing 10 cocoa seeds that have been sterilized in a 9 cm petri dish that had been overgrown by endophytic fungus colonies of potential isolates. Furthermore, cocoa seed was incubated and germination process was observed for one week.

If cocoa seeds normally germinated on candidate isolates of endophytic fungus then these isolates were classified as endophytic fungi. Conversely, if the germination was inhibited, or the cocoa seeds become rotten or drying, then the isolates were not endophytic fungi (Asniah *et al.*, 2014). Furthermore, endophytic fungi were identified with the aid of the Identification Book of Barnett and Hunter (1988).

The observed variables were the percentage of cocoa seed germination on fungal isolates, carried out by observing the germination process and counting the normally germinated seeds. The measure used the formula of Wilia *et al.* (2011), namely: DK = Percentage of germination (%)

$$DK = \frac{A}{B} \times 100\ \%$$

A = Number of normally germinated seeds
B = Number of observed seeds

Results and discussion

Endophytic fungi of cocoa plants

Seven endophytic fungi were successfully isolated from cocoa plants of clones Sultra 1 and Sultra 2 from the Cocoa Station of Plantation Office of Southeast Sulawesi at sub district Konda, South Konawe. The seven fungus isolates derived from the cocoa plant petiole and leaf is shown in Table 1.

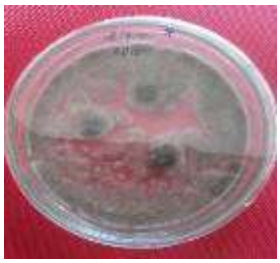
Table 1. Endophytic fungi isolated from cocoa plants.

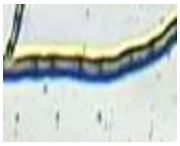
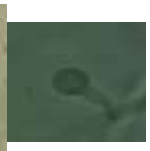
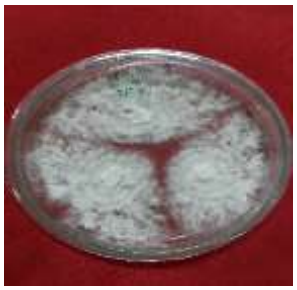
Plant tissues origin	Number of isolates	Spesies	Isolate codes
Leaf	1	*Aspergillus*sp.	ED5
Petiole	6	*Paecilomyces* sp.	EP3
		Paecilomyces sp.	EP1
		Cladosporium sp.	EP2
		Nigrospora sp.	EP4
		Paecilomyces sp.	EP6
		Paecilomyces sp.	EP8

The morphology and microscopic characters represented by each of these isolates can be seen in Table 2.

This study succeeded in isolating seven potential isolates of endophytic fungi from petiole and leaf of cocoa plants. The seven isolates were four isolates of *Paecilomyces* sp., one isolate of *Aspergillus* sp., one isolate of *Cladosporium* sp., and one isolate of *Nigrospora* sp. (Table 2). The four isolates of *Paecilomyces* sp. had different morphological characters of mycelia or colonies on PDA medium. It was suspected that the four isolates were distinct species; nevertheless it needs further identification to ensure the exact species of each isolate. The fungus *Paecilomyces* sp. was endophytic fungus that had been found in cacao plant. Hanada *et al.* (2010) managed to isolate some endophytic fungi from cacao plants, and that included *Paecilomyces* sp.

Table 2. Morphology and microscopic characteristics of endophytic fungi originated from cocoa plants.

Endophytic fungi	Microscopic	Morphology and microscopic characteristics
Aspergilus sp. 		- The mycelium is considered greyish white - Hyphae is sectional - Rounded spores - Conidiophores are straight, long, not insulated - Ends swell forming a vesicle
Paecilomyces sp. isolat EP3 		- The mycelium is considered yellowish white and clearly visible, - Spiny surface - Hyphae is sectional - Rounded spores - Unbranched
Paecilomyces sp. isolat EP1 		- The mycelium is considered yellowish white with clear concentric, the mycelium is thick - Spiny surface - Hyphae is sectional - Rounded spores with serrated edges
Cladosporium sp. 		- The mycelium is considered grayish white, not concentric - Hyphae is sectional - Rounded spores

Nigrospora sp. 	  	- The mycelium is considered white with yellowish edges, not concentric - Hyphae is sectional and shorth between cells - Single spores/conidia, uniseluler, rounded and slightly dark
Paecilomyces sp. isolat EP6 	  	- The mycelium is considered white, thick, grayish, and concentric - Hyphae is sectional - Rounded spores/conidia, with serrated edges
Paecilomyces sp. isolat EP8 	  	- The mycelium is considered white, slightly yellow and thin, clearly concentric but falters - Hyphae is sectional - Rounded spores/conidia, with serrated edges

Similarly, the endophytic fungus *Cladosporium* sp., which had been found in this study, has also been reported by Rubini *et al.* (2005), and it is potential as a biological agent to control the vascular streak dieback (VSD) disease. The abundance of endophytic fungi in various crops had been reported by many researchers (Daeley *et al.*, 2009; Hanada *et al.*, 2010; Amin *et al.*, 2014; Asniah *et al.*, 2014; Wahab *et al.*, 2016), including the existence of *Nigrospora* sp (Shekhawat *et al.*, 2010). Endophytic fungus *Nigrospora* sp. which was isolated had morphological characters of mycelium color, hyphae, spores or conidia form in accordance with the identification book. This identification was similar with previous study by Shekhawat *et al.* (2010), which found the endophytic fungus genus *Nigrospora* sp. isolated from the plant *Melia azedarach* L. with white mycelium color but a bit darker on the edges, insulated hyphae, round spores or conidia, single and unicellular. Asniah *et al.* (2009) managed to isolate the endophytic fungus *Nigrospora* sp., native of grasses and was able to control root diseases on broccoli crops.

Slightly different results, however, had previously been reported regarding the determination of *Aspergillus* sp. as endophytic fungus. Amin *et al.* (2014) had classified *Aspergillus* sp., isolated from the cocoa plant, as endophytic fungus and had potential as a biological control agent. Similarly, Kalyanasundaram *et al.* (2015) found that the endophytic fungus *Aspergillus terreus* produced

antibacterial compounds that had activity against bacterial infection characterized by the presence of high resistant zone (11 mm) against *Staphylococcus typhi* on nutrient agar medium (NA). While the results of this study found that *Aspergillus* sp. was not characterized as endophytic fungus based on the test results of cocoa seed germination.

The cocoa seeds did not germinate normally even the seeds were finally death (Table 3). Presumably, the differences were caused by various factors such as environmental conditions, cocoa clones used, possibly different species of *Aspergillus* sp., or other factors that can not be fully answered in this study.

Table 3. Germination test of cocoa seeds by endophytic fungi.

Endophytic fungi	Percentage of seeds germinated	Characteristic of cocoa seeds	Growth response of cocoa seeds
Control (without isolate)	90%	Non Pathogenic	Germinated
Aspergillus sp.	0%	Pathogenic	Not germinated
Paecilomyces sp. isolat EP3	90%	Non Pathogenic	Germinated and rooted
Paecilomyces sp. isolat EP1	100%	Non Pathogenic	Germinated and rooted
Cladosporium sp.	90%	Non Pathogenic	Germinated
Nigrospora sp.	60%	Non Pathogenic	Germinated
*Paecilomyces*sp. isolat EP6	50%	Non Pathogenic	Germinated
Paecilomyces sp. isolat EP8	50%	Non Pathogenic	Germinated and rooted

Stimulation test of cocoa seed germination

Endophytic fungus test on cacao seeds was conducted to determine whether the fungi were patogenic or non patogenic to cacao seeds. The observation of the percentage of seed germination was done with a simple tabulation, presented in

Table 3. The percentage of germination of cocoa seeds on endophytic fungal isolate EP1 (*Paecilomyces* sp.) was 100%, followed by *Cladosporium* sp. (90%), *Nigrospora* sp. (60%), *Paecilomyces* sp. EP6 isolate (50%) and *Paecilomyces* sp. EP8 isolate (50%). Meanwhile, *Aspergillus* sp. did not successfully stimulate the germination of cocoa seeds, so it was strongly suspected that the isolate was not endophytic fungus.

Co-evolution between endophytic fungi and host plants has occurred in long period of time that its presence does not cause symptoms on host plants they inhabit. The results of this study indicated that endophytic fungi *Paecilomyces* sp isolate EP1 and *Cladosporium* sp. can live in plant tissues without causing cocoa plants showing symptoms of illness, proved that cocoa seeds still germinated 100% and 90%.

The ability to stimulate germination by endophytic fungus was caused by the synthesis of secondary metabolites such as growth hormone auxin or antimicrobial compounds. The advantages of fungus endophyte to the plants are through the promotion of plant growth (Dai *et al*., 2008), increasing in plant resistance to multi-infections by pathogens (Malinowski *et al*., 2004), producing phytoalexin (Gao *et al*., 2010), protecting plants from infection and insects (Vega *et al*., 2008). Dai *et al*. (2008) further elaborated that the plants treated with endophytic fungi grew better on all observed variables, plant biomass was two times higher, and the content of indole acetic acid (IAA) and gibberllin accumulated higher than the control plants. Lu *et al*. (2000) reported that the endophytic fungus *Colletotrichum* sp., inside the plant *Artemisia annua* produced IAA to encourage the growth process.

Paecilomyces sp. EP1 isolate stimulated the percentage of cocoa seed germination by 100%. It was suspected that the endophytic fungus *Paecilomyces* sp. EP1 isolate produced growth hormone auxin, or in more quantity compared to other isolates, so that it could better stimulate the germination of the cocoa seeds in the PDA medium.

Srivastava (2002) described that the concentration of auxin can influence the growth of seed radicles. Endophytic fungi isolated in this study were therefore promising to be used as biological agents in controlling VSD pathogens on seed levels and in the field.

Conclusion

It has been successfully isolated and identified six isolates of endophytic fungi from cocoa plants, based on morphological characters. These isolates are *Paecilomyces* sp. EP1 isolate, *Paecilomyces* sp. EP3 isolate, *Paecilomyces* sp. EP6 isolate, *Paecilomyces* sp. EP8 isolate, *Cladosporium* sp. and *Nigrospora* sp. Endophytic fungus *Paecilomyces* sp. EP1 isolate is endophytic fungus which has the ability to stimulate the germination of cocoa seeds by 100%, followed by *Nigrospora* sp. (90%). The fungus *Aspergillus* sp., originated from the cocoa plants, can not be categorized as an endophytic fungus.

Acknowledgements

This study was part of a Cooperative Research Partnership and National Agricultural Development (KKP3N) program, financed by the Research and Development Fund of the Ministry of Agriculture of the Republic of Indonesia, with Contract Number: 54.32/HM.240/I.1/3/2016.K, dated, 7 March 2016. Thank was also extended to Dr. Teguh Wijayanto, for subtantial supports in editing and reviewing the manuscript.

Reference

Amin N, Salam M, Junaidi M, Aswan, Baco MS. 2014. Isolation and identification of endophytic fungi from cocoa plant resistant VSD M.05 and cocoa plant Susceptible VSD M.01in South Sulawesi, Indonesia. International Journal of Current Microbiology and Applied Science **3(2)**, 459-467. http://www.ijcmas.com/vol-3-2/Nur Amin, *et al*.pdf

Askindo. 2008. Discussion Report on VSD. Report was presented at a National Meeting "A Day Discussion: Crass Program Addressing VSD Disease", 5 June 2008. Clarion Hotel, Makassar.

Azhar I, Lee MT. 2004. Perspective for cocoa cultivation in Malaysia: Re-Look at the economic indicators. Malaysian Cocoa Journal **1,** 1-18.

Asniah. 2009. Potential of fungi from grassroots, sedges and bamboo rootings for disease control of "Gada" clubroot disease of Broccoli. Postgraduate Thesis, Bogor Institute of Agriculture, Bogor, Indonesia.

Asniah, Lestari D, Darlian L, Mariadi. 2014. Potential of nonpathogenic endophytic fungi from pepper crops (*Capsicum annuum* L.) as biofungicides of pathogen *Fusarium oxysporum*. Jurnal Agroteknos **24(2)**, 208-213.

Bailey BA, Strem MD, Wood D. 2009. *Trichoderma* species form endophytic associations within *Theobroma cacao* trichomes. Mycology Research **113**, 1365-1376. http://dx.doi.org/10.1016/j.mycres.2009.09.004

Barnet HL, Hunter BB. 1998. Illustrated Genera of Imperfect Fungi, 4ed. The American Phytopathological Society St. Paul Minnesota. APS Press. USA.

CABI. 2016. Improved management strategies for cocoa in Papua New Guinea. Accessed on 17 August 2016 http://www.cabi.org/projects/project/33019

Clay K. 1992. Fungal endophytes of tree leaves. *In*: Andrews JH, Hirano SS (eds). Microbial Ecologyof Leaves, Berlin: Springer Verlag, 341 – 349.

Dai C, Yu B, Li X. 2008. Screening of endophytic fungi that promote the growth of *Euphorbia pekinensis*. African Journal of Biotechnology **7(19),** 3505–3510.

Dinas Perkebunan dan Hortikultura. 2013. Statistik Perkebunan. Dinas Perkebunan dan Hortikultura Provinsi Sulawesi Tenggara, Kendari.

Dinas Perkebunan dan Hortikultura. 2015. Statistik Perkebunan. Dinas Perkebunan dan Hortikultura Provinsi Sulawesi Tenggara, Kendari

Dirjen Perkebunan. 2014. Statistik Perkebunan Indonesia Komoditas Kakao 2013-2015. Direktorat Jenderal Perkebunan. 61 hal.

Duoanla-Meli C, Langer E, Moufalo FT. 2013. Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. Fungal Ecology **6**, 212–222. http://dx.doi.org/10.1016/j.funeco.2013.01.004.

Gao FK, Dai CC, Liu XZ. 2010. Mechanisms of fungal endophytes in plant protection against pathogens. African Journal of Microbiology Research **4(13)**, 1346-1351. http://www.academicjournals.org/article/article1380280021_Gao *et al*.pdf

Guest D, Keane PJ. 2007. Vascular Streak Dieback: A new encounter disease of cacao in Papua New Guinea and Southeast Asia caused by the obligate Basidiomycete *Oncobasidium theobromae*. Phytopathology **97(12),** 1654 – 1657. http://dx.doi.org/10.1094/PHYTO-97-12-1654.

Halimah, Sri-Sukamto. 2007. Intensity of VSD disease on a number of cocoa clones, collections of Center for Coffea and Cocoa Research, Indonesia. Pelita Perkebunan **23**, 118–128.

Hanada RE, Pomella AWV, Costa HS, Bezerra JL, Loguercio LL, Pereira JO. 2010. Endophytic fungal diversity in *Theobroma cacao* (cocoa) and *T. grandiflorum* (cupuacu) trees and their potential for growth promotion and biocontrol of black-pod disease. Journal Biology **114,** 901-910. https://dx.doi.org/10.1016/j.funbio.2010.08.006

Ibiremo OS, Akanbi OSO, Oloyede AA, Adewale BD. 2014. Comparative evaluation of NPK fertilizer sources on the growth and yield of cocoa in Ibadan, Nigeria. World Journal of Biological Science **2(3)**, 062-066.

ICCO. 2013. Quarterly bulletin on Cocoa Statistics, **38(1),** Cocoa year 2011/2012. (Diunduh, 28 Januari 2016). http://www.icco.org/statistics/quarterlybulletincocoa-statistics.html.

Kalyanasundaram IJ, Nagamuthu, Muthukumaraswamy S. 2015. Antimicrobial activity of endophytic fungis isolated and identified from salt marsh plant in Vellar Estuary. Journal of Microbiology and Antimicrobials **7(2),** 13-20. http://dx.doi.org/10.5897/JMA2014.0334

Lu H, Zou WX, Meng JC, Hu J, Tan RX. 2000. New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant Science **151**, 67-73. http://dx.doi.org/10.1016/S0168-9452(99)00199-5

McMahon P, Purwantara A, Susilo AW, SriSukamto, Wahab A, Purung H, Hidayat M, Darna I, Taproni Smilja L, Guest D, Keane PJ. 2010. On-farm selection for quality and resistance to pest/diseases of cocoa in Sulawesi: (ii) quality and performance of selections against *Phytophthora* pod rot and Vascular-streak dieback. International Journal of Pest Management **56(4),** 351-361. http://dx.doi.org/10.1080/09670874.2010.503284

Purung H. 2008. The distribution of VSD in Sulawesi. Mars Cocoa Clinic. Palopo.

Rosmana A, Sylvia S, Syamsul B. 2010. Spraying applications on soil and leaves of fungicide Flutriafol to control VSD disease on cocoa plants. Seminar Proceedings and Annual Meeting XX of PEI and PFI, South Sulawesi.

Rosmana A, Papalangi IP, Kannapadan S, Rahim MD, Asman Nasaruddin. 2014. Cultural and pathologenic characterization of *Fusarium* fungi isolated from dieback branches of cacao. International Journal of Current Research and Academic Review **2(10)**, 1-6. http://www.ijcrar.com/vol-210/AdeRosmana,etal.pdf

Rubini MR, Ribeiro RTS, Pomella AWV, Maki CS, Araujo WL, dos Santos DR, Azevedo JL. 2005. Diversity of endophyte fungal community of cacao (*Theobromae cacao* L.) and biological control of *Crinipelis pernicosa*, causal agent of Witches Broom disease. International Journal of Biological Sciences **1,** 24-33.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1140355/

Samuel SGJ, Ismaiel A, Rosmana A, Junaid M, Guest D, McMahon P, Keane P, Purwantara A, Lambert FS, Rodriguez-Carresg M, Cubetag MA. 2012. *Vascular Streak Dieback* of cacao in Southeast Asia and Melanesia: in planta detection of the patogen and a new taxonomy. Journal of Fungal Biology **116(1),** 11-23.
http://dx.doi.org/10.1016/j.funbio.2011.07.009

Shekhawat KK, Rai DV, Batra A. 2010. Morphological study of endophytic fungi inhabiting leaves of *Melia azedarach* L. International Journal of Pharmaceutical Sciences Review and Research **5(3),** 177-180.

Simarmata, Rumilla. 2007. Isolation of endophytic microbes from medicinal plant *Gynura Procumbens* and its potential analysis as antimicrobial agent. Journal Penelitian Hayati **13**, 85-90.

Srivastava LM. 2002. Plant Growth and Development, Hormones and Environment. Academic Press, Orlando.

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA. 2008. Entomopathogenic fungal endophytes. Biological Control **46,** 72–82.
http://dx.doi.org/10.1016/j.biocontrol.2008.01.008

Wahab A, Wijayanto T, Taufik M, Bande LS, Gusnawaty, Asaad M, Rahim MD, Firmansyah AP. 2016. Role of biological agents and cocoa clones to control vascular streak dieback disease (*Ceratobasidium theobromae* Tallbot and Keane) of cocoa plants. International Journal of Biosciences **9(3),** 1-11.
http://dx.doi.org/10.12692/ijb/9.3.1-11