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INFLUENCE OF CULTURE MEDIUM ON THE SPORULATION AND VIABILITY OF *Aspergillus* spp. AND *Talaromyces* spp. ENTOMOPATHOGENIC FUNGI

Yuyun Fitriana[1], Radix Suharjo[1], I Gede Swibawa[1], Purnomo[1], Puji Lestari, & Eryka Merdiana[2]
[1] Department of Plant Protection, Faculty of Agriculture, University of Lampung, Lampung, Indonesia
[2] Department of Agrotechnology, Faculty of Agriculture, University of Lampung, Lampung, Indonesia
Jl. Sumantri Brodjonegoro No 1 Bandar Lampung 35145
E-mail: yuyun.fitriana@fp.unila.ac.id

ABSTRACT

Influence of Culture Medium on the Sporulation and Viability of* Aspergillus *spp. and* Talaromyces *spp. Entomopathogenic Fungi. The purpose of this study was to determine the effect of three kinds of cultures media on the spore production and viability of *Aspergillus* spp. (AS1, 6, 7, 9) and *Talaromyces* spp. (AS2–5, 8, 10) entomopathogenic fungi. This study was arranged using Factorial-Completely Randomized Design (CRD) with 2 factors and 3 replications. The first factor was three kinds of cultures media (potato dextrose agar (PDA), corn meal agar (CMA), and sabouraud dextrose agar (SDA)) and the second one was isolates of *Aspergillus* spp. or *Talaromyces* spp.. Data of spore production and spore viability were tested using ANOVA and if there was significantly difference, the data then further analyzed using Tukey's Honestly Significant Difference (HSD) test at 5% of significant level. The spore production of *Aspergillus* spp. were in the range of 0.58 - 14.27 x 10^8 spores mL^{-1} (PDA); 0.28 – 2.68 x 10^8 spores mL^{-1} (SDA) and 1.85 - 5.33 x 10^8 spores mL^{-1} (CMA). The highest spore production was achieved by AS1 isolate that was grown on PDA media. The spore produced by *Talaromyces* spp. were in the range of 2.15 – 28.62 x10^8 spores mL^{-1} (PDA); 0.28 – 29.43 x10^8 spores mL^{-1} (SDA) and 1.88 – 16.63 x10^8 spores mL^{-1} (CMA). The highest spore production was produced by AS8 isolate which were cultured on PDA. The spore viability among isolates of the two entomopathogenic fungi were not significantly different. The spore viability of *Aspergillus* spp. was in the range of 95.10 – 97.66% (PDA), 94.02 – 98.45% (SDA) and 92.86 – 98.20% (CMA). The spore viability of *Talaromyces* spp. was in the range of 95.83 – 100% (PDA), 85.83 – 100% (SDA), and 90.75 – 100% (CMA). Culture medium influenced spore production but not the spore viability. The best culture media used for spore production of both of the entomopathogenic fungi was PDA media.

Key words: *Aspergillus* spp., culture medium, entomopathogenic fungi, spore production and viability, *Talaromyces* spp.

ABSTRAK

Pengaruh Medium terhadap Sporulasi dan Viabilitas* Jamur Entomopatogen Aspergillus *spp.* dan Talaromyces *spp. Penelitian ini bertujuan untuk mengetahui pengaruh tiga jenis media tumbuh terhadap produksi dan viabilitas spora jamur *Aspergillus* spp. (AS1, 6, 7, 9) dan *Talaromyces* spp. (AS2–5, 8, 10). Penelitian ini disusun menggunakan Rancangan Acak Lengkap Faktorial (RAL) dengan 2 faktor dan 3 ulangan. Faktor pertama adalah tiga jenis media tumbuh (*potato dextrose agar* (PDA), *corn meal agar* (CMA), dan *sabouraud dextrose agar* (SDA)) dan faktor kedua adalah isolat jamur *Aspergillus* spp. atau *Talaromyces* spp .. Data produksi dan viabilitas spora diuji menggunakan sidik ragam dan jika terdapat beda nyata, diuji lanjut menggunakan uji Beda Nyata Jujur (BNJ) pada taraf nyata 5%. Produksi spora *Aspergillus* spp. berada dalam kisaran 0,58 - 14,27 x 10^8 spora mL-1 (PDA); 0,28 - 2,68 x 10^8 spora mL^{-1} (SDA) dan 1,85 - 5,33 x 10^8 spora mL^{-1} (CMA). Produksi spora tertinggi dihasilkan oleh isolat AS1 yang ditumbuhkan pada media PDA. Produksi spora *Talaromyces* spp. berada dalam kisaran 2,15 - 28,62 x10^8 spora mL^{-1} (PDA); 0,28 - 29,43 x10^8 spora mL^{-1} (SDA); dan 1.88 - 16,63 x10^8 spora mL^{-1} (CMA). Produksi spora tertinggi dihasilkan oleh isolat AS8 yang ditumbuhkan pada media PDA. Viabilitas spora masing masing isolat dari kedua jamur tersebut tidak berbeda nyata. Viabilitas spora *Aspergillus* spp. berada dalam kisaran 95,10 - 97,66% (PDA), 94,02 - 98,45% (SDA) dan 92,86 - 98,20% (CMA). Viabilitas spora *Talaromyces* spp. berada di kisaran 95,83 - 100% (PDA), 85,83 - 100% (SDA), dan 90,75 - 100% (CMA). Media tumbuh secara nyata mempengaruhi produksi spora tetapi tidak untuk viabilitas spora. Media tumbuh terbaik untuk produksi spora kedua jamur tersebut adalah media PDA.

Kata kunci: *Aspergillus* spp., jamur entomopatogen, media tumbuh, produksi dan viabilitas spora, *Talaromyces* spp.

Entomopathogenic fungi is one of the promising biocontrol agents for controlling plant pest insects (Shah & Pell, 2003; Khan et al., 2012). Some species of entomopathogen such as *Beauveria bassiana* (Balsamo), Vuillemin (Zimmermann, 2007a) and *Metarhizium anisopliae* (Metschinikoft) (Zimmermann, 2007b) have been proven as excellent biological control agents against large number of pest insects. Recently, a group of fungi belongs to the genus Aspergillus have also been reported as entomopathogen (Devi et al., 2017). This fungi had been reported to cause mortality many kinds of pest insects (Shubha et al., 2014; Yang et al., 2015; Hamdani et al., 2011; Pasaru et al., 2014; Bordoloi et al., 2012). Another group of fungi, namely Talaromyces, have also been proven as potential biological control agent. The fungus showed good capability to inhibit growth of some plant pathogens, such as *Verticillium dahliae* (Bahramian et al., 2016), *Fusarium oxysporum* (Barahmian et al., 2016), *Gaeumannomyces graminis* var *tritici* (Ghanbari & Mohammadi, 2015), *Sclerotinia sclerotiorum* (McLaren et al., 1994) and nematodes such as *Pratylenchus oryzae* (Kisaakye, 2014). In our previous study, we found fungi from the genus of Aspergillus and Talaromyces which had capability to cause death of cocoa mirid bugs (*Helopeltis* spp.).

For field applications, large-scale propagation of entomopathogenic fungi is a necessary step that must be performed. Before mass production, selection of growing media (agar medium) for starter of entomopathogenic fungi is one of the most important things to carry out. Several types of culture medium commonly used for entomopathogenic fungi, such as sabouraud dextrose agar (SDA), potato dextrose agar (PDA), and corn meal agar (CMA) (Ingle, 2014; Senthamizhlselvan et al., 2010; Ali et al., 2016). Those three above mentioned media has different ingredients that may give different effect to different entomopathogenic fungi. Many reports stated that different entomopathogenic fungi will not perform similar growth in the same medium (El Damir, 2006; Pandey & Kanaujia, 2006; Francisco et al., 2006, Hase & Nasreen, 2017). This study was performed to investigate effect of those three mentioned media (PDA, SDA and CMA) on the growth, spore production and spore viability of *Aspergillus* spp. and *Talaromyces* spp. entomopathogenic fungi. The result of this study will give information on the suitable culture medium that can be used for cultivation of *Aspergillus* spp. or *Talaromyces* spp..

MATERIALS AND METHOD

Study site. This study was performed from March to June 2017. Fungal propagation and observation on their spore production and spore viability were conducted in the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, University of Lampung.

Fungal isolates. Four isolates of *Aspergillus* spp. and six isolates of *Talaromyces* spp. entomopathogenic fungi were used in this study. The isolates were obtained from three kinds of plant rhizosphere, namely corn, pineapple and chili. They were morphologically and molecularly identified using ITS1 and ITS4 (Unpublished data) (Table 1).

Table 1. Isolates of entomopathogenic fungi used in this study

Isolate	Isolated from (rhizosphere)	Origin	Year Isolated	Identity
AS 1	Pineapple	Lampung Tengah	2015	*Aspergillus* sp.
AS 2	Pineapple	Lampung Tengah	2015	*Talaromyces* sp.
AS 3	Pineapple	Lampung Tengah	2015	*Talaromyces* sp.
AS 4	Pineapple	Lampung Tengah	2015	*Talaromyces* sp.
AS 5	Pineapple	Lampung Tengah	2015	*Talaromyces* sp.
AS 6	Corn	Lampung Selatan	2016	*Aspergillus* sp.
AS 7	Corn	Lampung Selatan	2016	*Aspergillus* sp.
AS 8	Corn	Pesawaran	2016	*Talaromyces* sp.
AS 9	Corn	Pesawaran	2016	*Aspergillus* sp.
AS 10	Chili	Unknown	2017	*Talaromyces* sp.

Culture medium. All the isolates were grown in sterile plastic petri dishes (90-mm diameter) contains three kinds of media i.e potato dextrose agar (PDA; HIMEDIA® India), sabouraud dextrose agar (SDA; HIMEDIA® India) and corn meal agar (CMA; HIMEDIA® India). The inoculated petri dishes were incubated at 27 ± 1°C for 7 days.

Culture preparation. Mycelial plugs (4-mm diameter) of each isolates were excised from the margins of colonies (2-days-old cultures that were incubated at 27 ± 1 °C) and placed in the center of a sterile plastic petri dish (90-mm diameter) containing 30 mL of media (PDA, SDA or CMA). Three replicates were prepared for each treatment.

Preparation of the spore suspension. Spore suspension was prepared using sterile 0.1% Tween 80. As much as 10 mL of sterile 0.1% Tween 80 was added into sterile plastic petri dish containing 7-days-old entomopathogenic fungal isolates and scraping the mycelium from plate cultures carefully. The suspension then filtered using sterile filter funnel (0.2-mm of mesh size) to remove mycelia and placed into sterile erlenmeyer flask (50 mL of volume).

Estimating spore production. One mL of spore suspension was placed into haemocytometer. The spore production was counted using a haemocytometer under binocular microscope (Leica, *Switzerland*) with 400x of magnification. Observation was performed on the presence of individual spore on 5 square grids of haemocytometer (medium size). Data of spore produced by each isolates of entomopathogenic fungi was average of individual spore observed on 5 square grids. Total spore production was analyzed using formula described by Syahnen et al. (2014) as follows; S = R x K x F; S = spore production, K = a constanta (2.5×10^5), F = dilution factor used.

Spore germination. Spore suspension (25 µL; 1×10^6 spore mL^{-1}) from each isolates were placed individually (3 inoculation point) into a sterile plastic petri dish (9-mm diameter) containing 30 mL each of agar media. Each inoculation point was covered with a sterile glass coverslip (18 mm × 18 mm). The dishes were incubated at 27 ± 1 °C for 10 h. Total spore geminated were calculated under binocular microscope (Leica, *Switzerland*) with 400x magnification. Spores determined to be germinated if the length of the fur is 2x length of conidia diameter (Espinel-Ingroff, 2000).

Experimental design and statistical analysis. This study was arranged using Factorial-Completely Randomized Design (CRD) with 2 factors and 3 replications. The first factor was three kinds of cultures media, namely PDA, CMA and SDA. The second factor was isolates of *Aspergillus* spp. or *Talaromyces* spp.. Data transformations was carried out to create nearly equal spreads or additive relationship of the data. Spore production and spore viability data were analyzed using ANOVA and if there was significantly different, the data was further investigated using Tukey's Honestly Significant Difference (HSD) test at 5% of significant level.

RESULTS AND DISCUSSION

Four isolates of *Aspergillus* spp. and six isolates of *Talaromyces* spp. entomopathogenic fungi (Figure 1) were investigated on their spore production and spore viability cultivated on three kinds of cultures media, namely potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and corn meal agar (CMA). Those isolates showed the capability to infect and cause mortality of cocoa mirid bugs (*Helopeltis* spp.) (Unpublished data).

Spore production. Spore production among *Talaromyces* spp. isolates and *Aspergillus* spp. isolates was significantly different. The spore generated by both *Aspergillus* spp. and *Talaromyces* spp. was significantly influenced by the cultures media used as well as the isolates (Table 2).

***Aspergillus* spp..** Spore production of each isolate was in the range of 0.58 – 14.27 x 10^8 spores mL^{-1} (PDA); 0.28 – 2.68 x 10^8 spores mL^{-1} (SDA) and 1.85 – 5.33 x 10^8 spores mL^{-1} (CMA). The highest spore production was achieved by AS1 isolate grown on PDA media, and it was significantly higher than AS1 isolate on SDA and CMA or all isolates which were cultured on PDA, SDA and CMA. The lowest spore production was obtained by AS6 isolate on SDA media and it was statistically not different from AS7 isolate that was cultured on PDA media also AS 1 and AS9 isolates grown on SDA media (Table 3).

Based on the data of average of spore production by all isolates in the three cultures medium, the highest spore production was obtained by isolates that were grown on PDA media (8.78 x 10^8 spore mL^{-1}) followed by CMA (3.28 x 10^8 spore mL^{-1}) and SDA media (1.23 x 10^8 spores mL^{-1}) (Figure 2).

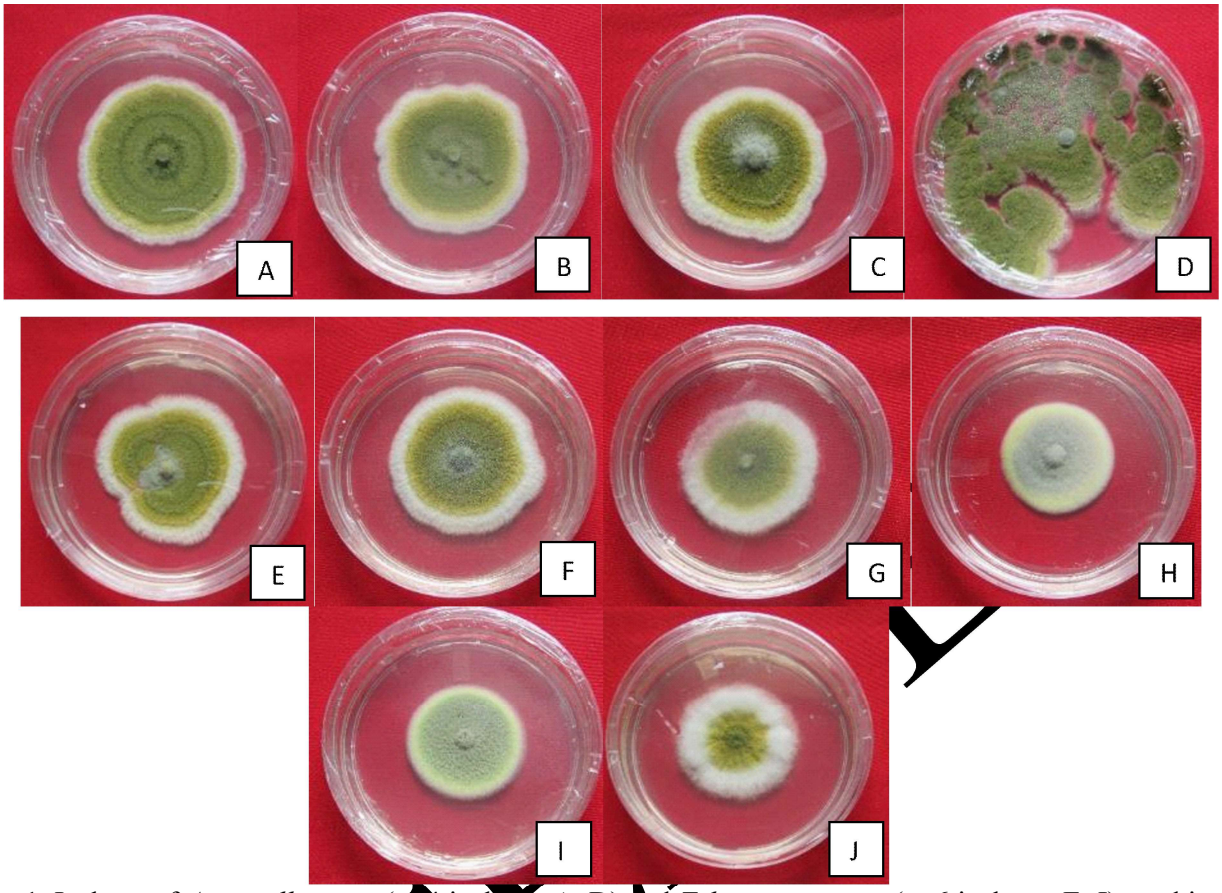

Figure 1. Isolates of *Aspergillus* spp. (n=4 isolates; A–D) and *Talaromyces* spp. (n=6 isolates; E–J) used in this study. A. AS1, B. AS6, C. AS7, D. AS9, E. AS2, F, AS.3, G. AS4, H. AS5, I. AS8, J. AS10

Table 2. Analysis of variance of spore production of *Aspergillus* spp. and *Talaromyces* spp.

Source	df	Anova ss	Mean square	Fvalue	Pvalue
		Aspergillus spp.			
Cultures media	2	15.12	7.56	203.78	<.0001
Isolates	3	2.20	0.73	19.78	<.0001
Cultures media * isolates	6	14.01	2.34	62.94	<.0001
		Talaromyces spp.			
Cultures media	2	8.44	4.22	163.82	<.0001
Isolates	5	72.15	14.43	560.22	<.0001
Cultures media * isolates	10	23.92	2.39	92.86	<.0001

***Talaromyces* spp..** Spore produced by all *Talaromyces* spp. isolates were in the range of 2.15 – 28.62 x 10^8 spores mL^{-1} (PDA); 0.28 – 29.43 x10^8 spores mL^{-1} (SDA); and 1.88 – 16.63 x10^8 spores mL^{-1} (CMA). The highest spore production was produced by AS8 isolate which were cultured on PDA and it was not significantly different with AS8 isolate grown in SDA and CMA media. This AS8 isolate was significantly different than the other isolates grown in the three cultures media. The lowest spore production was produced by AS4 cultured in SDA and it was not significantly different with AS2, AS3 and AS 10 grown in SDA (Table 4).

Based on the average of the spore production of all the isolates that were cultured in the three kinds of medium showed that the isolates which were grown in PDA media produced the highest spore (10.34 x10^8

Table 3. Spore production of *Aspergillus* spp. in three kinds of different cultures media

Media	Isolates	Spore production (x 10^8 spore/mL^{-1})
PDA	AS 1	14.27 (3.84) a
	AS 6	10.20 (3.26) b
	AS 7	0.58 (1.04) fg
	AS 9	10.05 (3.24) b
SDA	AS 1	1.12 (1.27) efg
	AS 6	0.28 (0.88) g
	AS 7	2.68 (1.78) de
	AS 9	0.83 (1.15) fg
CMA	AS 1	1.85 (1.53) def
	AS 6	5.33 (2.38) c
	AS 7	3.33 (1.96) cd
	AS 9	2.58 (1.75) de
Pvalue		<.0001
HSD 5%		0.57

Number in one column followed by the same letter (s) was not significantly different based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level. Number in parentheses were the result of transformation $\sqrt{x+0.5}$.

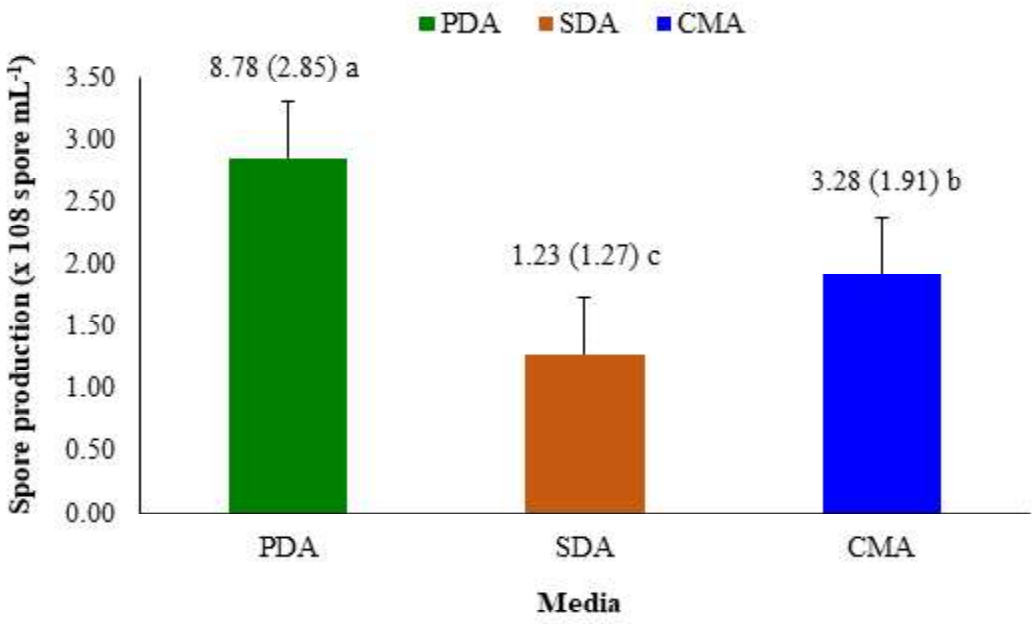

Figure 2. Average of spore production of *Aspergillus* spp. cultured in three kinds of different cultures media. The best spore production was obtained by isolates grown on PDA media, followed by CMA media and SDA media. Number in parentheses are the result of transformation $\sqrt{x+0.5}$ of spore production data. Number with the same letter was not significantly different based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level

spores mL^{-1}), followed by CMA (6.81 x10^8 spores mL^{-1}) and SDA media (6.52 x10^8 spores mL^{-1}) (Figure 3). SDA media has been reported as the best media for *Aspergillus* spp. followed by PDA media and the last was CMA media (Ali et al., 2016). Ingle (2014) noted that SDA media can provide colony growth and sporulation of entomopathogenic fungi *Nomuraea rileyi* better than PDA media. Senthamizhlselvan et al. (2010) revealed *B. bassiana* isolates BbMdKKL 2106 had maximum sporulation on SDA media (8.95 x 10^8 spore

Table 4. Spore production of *Talaromyces* spp. in three different cultures media

Media	Isolate	Spore production (x 10^8 spore mL^{-1})
PDA	AS 2	13.67 (3.76) bc
	AS 3	6.83 (2.71) d
	AS 4	2.15 (1.63) fg
	AS 5	7.43 (2.81) d
	AS 8	28.62 (5.40) a
	AS 10	3.33 (1.95) ef
SDA	AS 2	0.92 (1.18) ghi
	AS 3	1.00 (1.22) ghi
	AS 4	0.28 (0.88) i
	AS 5	7.10 (2.75) d
	AS 8	29.43 (5.47) a
	AS 10	0.40 (0.95) hi
CMA	AS 2	3.17 (1.91)ef
	AS 3	5.05 (2.35) de
	AS 4	1.50 (1.41) gh
	AS 5	1.88 (1.5[illegible] fg
	AS 8	16.63 (4.14)
	AS 10	12.60 (3.62) c
Pvalue		<[illegible]001
HSD 5%		0[illegible]49

Number in one column followed by the same letter (s) was not significantly different based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level. Number in parentheses were the result of transformation $\sqrt{x+0.5}$.

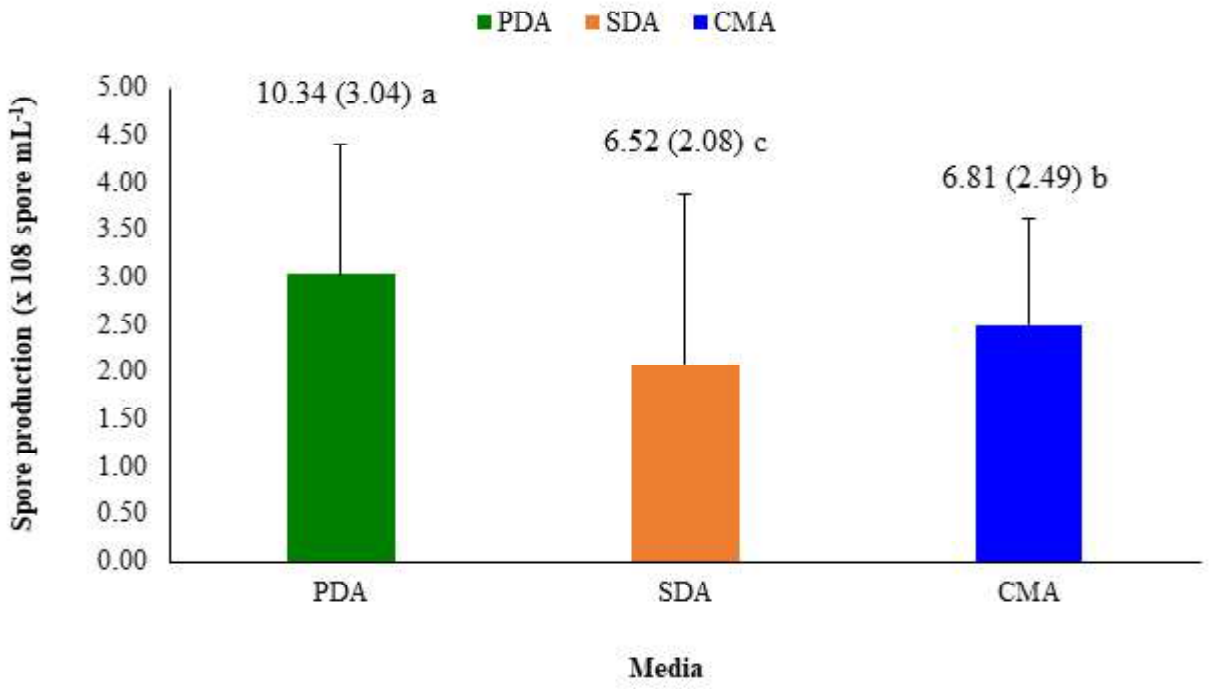

Figure 3. Average of spore production of *Talaromyces* spp. cultured in three different cultures media. The best spore production was obtained by isolates grown on PDA media, followed by CMA media and SDA media. Number in parentheses are the result of transformation $\sqrt{x+0.5}$ of spore production data. Number with the same letter was not significantly different based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level.

mL^{-1}) while FpCmKKL 1526 isolate had minimum sporulation on PDA media (0.28 x 10^8 spore mL^{-1}).

In this study, the isolates of *Aspergillus* spp. and *Talaromyces* spp. which were grown on PDA media were able to produce the highest spores followed by CMA and SDA (Figure 2; 3). It is suggested that PDA media is more suitable to optimize the spore production of *Aspergillus* spp. and *Talaromyces* spp. compared with SDA and CMA.

In line with this study, some reports also stated that fungal isolates grown on PDA media have improved their ability and showed better spore production than other media. Gupta et al. (2012) described that *Aspergillus niger* grown on PDA media showed better growth compared to CYA (Czapek's Dox + Yeast Extract Agar) and LCA (Lignocellulose Agar). PDA media are also reported to be able to produce better growth and sporulation of *Rhizoctonia solani*, *Uromyces appendiculatus*, *Cercospora beticola*, *Alternaria alternata*, *Alternaria helianthi* and *Aspergillus fumigatus* than Czapek's agar (CZA) media, CMA (corn meal agar), NA (nutrient agar) and SDA (sabouraud dextrose agar) (Hase & Nasreen, 2017).

The results of this study indicated that culture medium influenced spore production of *Aspergillus* spp. and *Talaromyces* spp.. All solates of both entomopathogenic fungi grown in three kinds of medium (PDA, SDA and CMA) produced spores which were significantly different (Tabel 2, 3, 4; Figure 2, 3).

Spore viability. Spore viability among all *Talaromyces* spp., as well as *Aspergillus* spp, was not significantly different. Each of the isolates produced spores with similar viability. The spore viability of both *Aspergillus* spp. and *Talaromyces* spp. was not influenced by the cultures media or the isolates (Table 5).

Table 5. Analysis of variance of spore viability of *Aspergillus* spp. and *Talaromyces* spp.

Source	df	Anova ss	Mean square	Fvalue	Pvalue
		Aspergillus spp.			
Cultures media	2	0.002	0.001	0.02	0.98
Isolates	3	0.14	0.05	0.91	0.45
Cultures media * isolates	6	0.13	0.02	0.43	0.85
		Talaromyces spp.			
Cultures media	2	0.49	0.24	3.07	0.06
Isolates	5	0.69	0.14	1.74	0.15
Cultures media * isolates	10	0.84	0.08	1.06	0.42

Table 6. Spore viability of *Aspergillus* spp. in three different cultures media

Media	Isolates	Spore viability (%)
PDA	AS 1	95.10
	AS 6	95.30
	AS 7	97.66
	AS 9	97.14
SDA	AS 1	94.02
	AS 6	96.67
	AS 7	98.45
	AS 9	95.24
CMA	AS 1	96.58
	AS 6	98.20
	AS 7	97.88
	AS 9	92.86
Pvalue		0.89 [nd)]
HSD 5%		0.29

[nd)] Not significantly different

***Aspergillus* spp..** The spore viability of *Aspergillus* spp. was in the range of 95.10 – 97.66% (PDA), 94.02 – 98.45% (SDA) and 92.86 – 98.20% (CMA) (Table 6). The highest average of spore viability produced by the isolates grown on CMA media (96.38%), PDA **S**media (96.30%) and the lowest on SDA media (96.10%) (Figure 4).

***Talaromyces* spp..** *Talaromyces* spp. showed spore viability in the range of 95.83 – 100% (PDA), 85.83 – 100% (SDA), and 90.75 – 100% (CMA) (Table 7). Based on the average of spore viability, the highest viability was obtained by isolate grown in PDA media (98.07%) followed by CMA (96.71%) and the lowest was SDA (93.74%) (Figure 5).

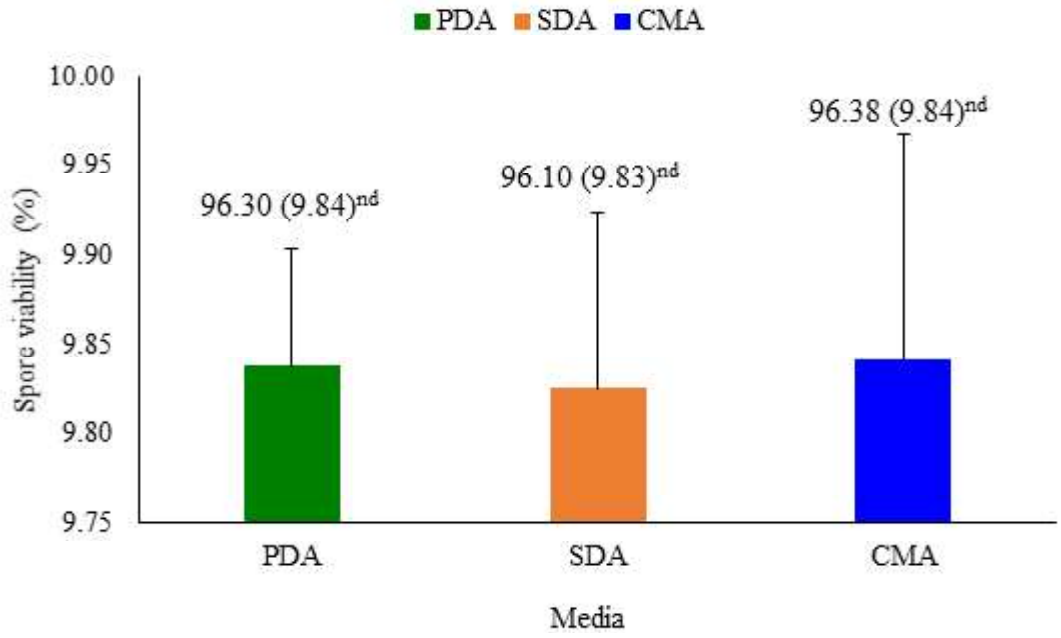

Figure 4. Spore viability of *Aspergillus* spp. cultured in three different cultures media. Number in parentheses are the result of transformation $\sqrt{x+0.5}$ of spore viability data. Spore viability of the isolates grown in the three kinds of different cultures media was not significantly different (nd) based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level

Table 7. Spore viability of *Talaromyces* spp. in three different cultures media

Media	Isolates	Spore viability (%)
PDA	AS 1	95.10
	AS 6	95.30
	AS 7	97.66
	AS 9	97.14
SDA	AS 1	94.02
	AS 6	96.67
	AS 7	98.45
	AS 9	95.24
CMA	AS 1	96.58
	AS 6	98.20
	AS 7	97.88
	AS 9	92.86
Pvalue		0.15 nd)
HSD 5%		0.40

nd) Not significantly different

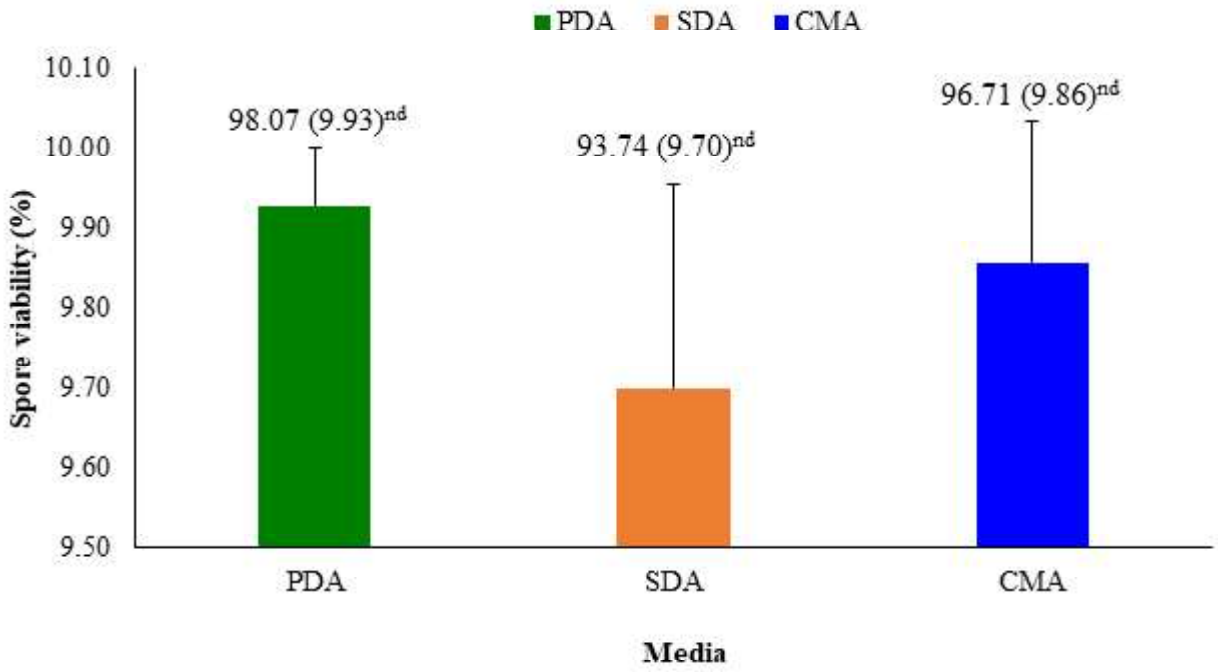

Figure 5. Spore viability of *Talaromyces* spp. cultured in three different cultures media. Number in parentheses are the result of transformation $\sqrt{x+0.5}$ of spore viability data. Spore viability of the isolates grown in the three kinds of different cultures media was not significantly different (nd) based on Tukey's Honestly Significant Difference (HSD) test at 5% of significant level.

The fact that spore viability is not affected by the type of media was also reported in *Verticillium lecanii*. Derakhshan et al. (2008) explained that *V. lecanii* grown on MYB (molasses yeast broth) media, PCB (potato carrot broth), JYB (jaggery yeast broth), SYB (sucrose yeast broth), PSB (potato sucrose broth) and PDB (potato dextrose broth) had a relatively similar spore viability, ranging from 89 to 95%.

CONCLUSIONS

Culture medium (PDA, SDA and CMA) influenced spore production of *Aspergillus* spp. and *Talaromyces* spp.. However, the medium did not affect the viability of spore produced by those two entomopathogenic fungi. Spore viability produced among all the isolate of the two entomopathogenic fungi were not significantly different. PDA was the best culture media used for spore production for both entomopathogenic fungi.

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