JURNAL RJPBSC VOL 8 NO. 3 2017.pdf

by Nurbailis Nurbailis

Submission date: 19-Feb-2018 03:02PM (UTC+0800)
Submission ID: 918052719
File name: JURNAL RJPBSC VOL 8 NO. 3 2017.pdf (157.27K)
Word count: 2816
Character count: 14557

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Viability and Temperature Effect to Conidia Germination of *Trichoderma* spp Indigenous Banana Rhizosphere in West Sumatera Indonesia

Nurbailis, Martinius, Ujang Khairul, Vera Amelia

Department of Plant Plant Protection, Faculty of Acriculture, AndalasUniversity, Padang 25163, Indonesia

ABSTRACT

The purpose of this research was to study the viability and temperature effect on conidial germination of *Trichoderma* spp. indigenous banana rhizosphere in west Sumatera Indonesia. The experiment used Completely Randomized Design (CRD) with 15 treatments and 4 replications. The treatments were 15 *Trichoderma* isolates from various banana rhizosphere in West Sumatera Indonesia and had evaluated in vitro and in planta for biological activity against *Fusarium oxysporum* f.sp *cubense*. The observations were : the width of colony, production of conidia, the germination of conidia and conidia sensitivity to temperature. The result showed that the viability of *Trichoderma* spp isolated from various banana rhizosphere in West Sumatera Indonesia were different. *Trichoderma* isolates : TD1, SO10, were the isolates that had better viability than other isolates and more tolerant to high temperature.

Keywords: *Trichoderma* spp, viability, temperature, conidia

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Trichoderma spp. are commonly found in all climatic zones, this fungi has habitat that dispersed in many kinds of soil and agriculture land. The most typical habitats of these fungi include soil and rotting wood [1,2]. Reference [3] reported that *Trichoderma* isolated from six different substrata and 49 localities in Poland, the highest diversity of *Trichoderma* species was detected in the set of 22 isolates originating from soil, which included nine species (*T. atroviride*, *T. citrinoviride*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. polysporum*, *T. tomentosum*, *T. viride*, *T. viridescens*).

According to [4] the colony of *Trichoderma* spp. were different for each isolate, depend of species and strain. They were yellowish, yellow, white , green colour. Isolate that has green colour indicated that producted the dense conidia. According to [5] the ability of *Trichoderma* to suppress the growth of pathogenic fungus was determined by conidia germination. Several previously researches proved that isolates of *Trichoderma* spp. had different abilities to suppress the growth of pathogenic fungus. Reference [6] reported four isolates of *Trichoderma* consisted of: three isolates were *T. viride* and one was *T.harzianum* from the tea ecosystem in North Bengal India indicated that *T. harzianum* was the best isolate in inhibiting the miselium growth of tea pathogens (*Cortisium invisum* and *Pestalotiopsis theae*). Reference [7] also observed that 33 *Trichoderma* isolates from several banana production center in West Sumatera, in planta condition showed that different abilities to suppress the growth of *Fusarium oxysporum* f.sp. cubense (Foc). There were several isolates of *Trichoderma* effectively against *Foc* caused Fusarium wilt disease of banana.

The difference of *Trichodema* isolates abilities to supress the growth of pathogens caused by the viability diversity of *Trichoderma* isolates and the tolerance of conidia germination to the temperature. According to [8], generally *T .harzianum* can grow well at 25-30°C on Potato Dextrosa Agar and increase the temperature at 37°C made reduce the growth and no growth was observed at 45°C after six days of inoculation. According to [9] *Trichoderma* spp from different agro climatic zone of India indicated that the ability of conidia to germinate of many isolates were reduced at 37^{0}C except *T. asperellum*-TaDOR67 and this isolate effectively controlled the collar rot disease caused by *Sclerotium rolfsii* in groundnut when screened *in vitro* and *in vivo*. [10] reported that The Maximum temperature for conidium production of *T. atroviride* occurred at 25°C after incubation during 20 days. Germination of conidia that produced at 30°C more rapidly than the other temperature and gave the better bioactivity in comparison with incubation at 20°C or. 25°C.

The purpose of this research was to study about the viability diversity and the effect of temperature changes to conidia germination of *Trichoderma* spp indigenous banana rhizosphere in west Sumatera that potential to suppress the growth of Foc.

METHODOLOGY

Design: This experiment used Completely Randomized Design (CRD) with 15 treatments and 4 replications. The treatments consisted of 15 *Trichoderma* isolates that isolated from various banana rhizosphere in West Sumatera Indonesia. All isolates had been evaluated in vitro and in planta for biological activity against *Fusarium oxysporum* f.sp *cubense* (Table 1, figure 1).

Table 1: *Trichoderma spp.* Indigenous Various of Banana Rhizosphere in West Sumatera Indonesia

No	Isolate	No	Isolate
1	TD 1	9	SO 9
2	TD 3	10	SO 10
3	TD 4	11	SO 11
4	TD 9	12	PP 1
5	TD 11	13	PP 4
6	TD 12	14	PP 6
7	SO 2	15	PP 7
8	SO 6		

ISSN: 0975-8585

TD = Isolate from Tanah Datar region; SO = Isolate from Solok region; PP = Isolate from Padang Pariaman region

Data had analyzed with ANOVA (Analisis of Variants) and followed by Duncan New Multiple Range Test (DNMRT) at 5% level.

Propagation of *Trichoderma* spp. Isolates

Source of *Trichoderma* inoculum was obtained from several banana production center in West Sumatera Indonesia which was collected in Phytopathology laboratorium, Faculty of Agricultural, Andalas university. The isolates were rejuvenated again on Potato Dextrose Agar (PDA) for 6 days.

Preparing the suspension of conidia

Trichoderma spp were propagated in petridish that contain 10 ml PDA medium, then incubated during 6 days. After that conidia were removed by adding 10 ml of sterile distilled water and 0.05% Tween 80 as a spreader in a petri dish. Conidia removed from medium by using a soft brush. The cocentration of conidia was calculated by haemocytometer. The concentration used for the treatment was 10^6 conidia ml^- suspension.

Viability of *Trichoderma* spp

The Width of colony, indicated by growing *Trichoderma* fungal mat in the petridish that contain 10 ml PDA medium and then incubated at room temperature (28^0C) The width of colony was mesuared 2 days after inoculation untill the petridish full well.

Evaluation of conidia germination. The conidia germination was determined with using slide culture. The method of slide culture : PDA medium with 1 cm^2 area and 0,5 cm thickness was placed on sterile object glass. Then Dropped with 10 µl of conidia suspension that contain 10^6 conidia ml^{-1} suspension, and then covered with cover glass. The slide culture was put in sterilized petridish which had been coated with whatman paper. Conidia germination was observed by using binocular microscope with magnifying 400x. Percentage of conidia germination was counted from 100 conidia. Conidia were clarified germinating of germination tube longer than diameter of conidia (figure 1) [11]

Production of Conidia. Production of conidia was determined in way, first,. each isolates of conidia suspension were prepared with the concentration 10^6 conidia ml^- suspension. then was took 0,1 ml and put in petridish that contain of PDA medium. The culture was incubated for 6 days at room temperature (28^0C). Subsequently conidia were removed by adding 10 ml of sterile distilled water and 0.05% Tween 80 as a spreader in a petri dish. Conidia removed from media by using a soft brush. The conidia suspension was put into Erlenmeyer flask and added 50 ml sterilized aquadest, vortexed for 5 minutes, filtered and diluted. Conidia concentration of each isolates was counted with haemocytometer.

Effect of Temperature changes to conidial germination

Conidia sensitivity to temperature was determined in way, conidia suspension was took 10 ml with concentration 10^6 conidia ml^- suspension from each isolates and then put into test tube. Each isolates were treated with 20^0C, 30^0C, 35^0C and 50^0C during 30 minutes using *waterbath*. After treatment, the conidia viability of each isolates were determined with the same method on evaluation of conidia germination.

RESULT AND DISCUSSION

Viability of *Trichoderma* spp.

The Width of Colony

Based on the analysis of variance of the colony width of *Trichoderma* spp. were significant among isolates (α 0,05). Four isolates were categorized as good of colony width : SO2, PP4, TD1 and SO9 with average 4552,50 – 5675,50 mm^2 (Table 2)

ISSN: 0975-8585

Table 2: The Width of *Trichoderma* spp colonies that Incubated during 3 days at 28^{0}C

The Kind of *Trichoderma* isolate	The width of colony(mm^2)
SO 2	5675.50 a
PP 4	5327.75 b
TD 1	4583.25 c
SO 9	4552.50 c
PP 7	4094.50 d
S0 10	4020.00 d
SO 6	3840.50 d e
TD 4	3652.50 e f
TD 3	3532.00 e f
PP 6	3417.50 f
TD 12	3098.75 g
SO 11	2910.50 g h
PP 1	2873.75 g h
TD 11	2709.50 h i
TD 9	2542.00 i
CV. 5.12%	

The figure in the column followed by the same lowercase letter are not significantly different according to DNMRT at 5% level

Four isolates of *Trichoderma* that identified the good growth were SO2, PP4, TD1 and SO9. Four isolates grew quickly, only 5 days could be able to complete the petridish. The isolate could grow quickly had a good competition againts pathogen. According to [12] four *Trichoderma* isolates from north Bengal in India indicated that *T. harzianum* was the best isolate in inhibiting the miselium growth of tea pathogens (*Cortisium invisum* and *Pestalotiopsis theae*).

Production of conidia

The amount of conidia production were different significantly among isolates (α 0,05). Four isolates were categorized as having height conidia production, those were TD1, TD3, SO10 and PP7 with average amount of conidia 3,20-3,69 x 10^{10} ml^{-} (Table 3)

Table 3: Conidia Production of *Trichoderma* spp that Incubated during 6 days at 28^{0}C

The Kind of Isolates	The Production of Conidia (ml^{-} suspension) (x 10^{10})
TD 1	3.69 a
TD 3	3.46 a b
S0 10	3.27 b
PP 7	3.20 b c
TD 9	3.15 b c
PP 1	2.87 c d
SO 6	2.74 d e
SO 11	2.55 d e f
TD 11	2.42 e f g
SO 9	2.19 f g
SO 2	2.16 g
PP 4	2.10 g h
PP 6	1.76 h i
TD 12	1.49 i j
TD 4	1.30 j
CV. 10.03%	

The figure 1 in the column followed by the same lowercase letter are not significantly different according to DNMRT at 5% level

Trichoderma isolates; TD1, TD3 and SO10 producted more conidia than the others. In this case the good growth of *Trichoderma* isolate not always indicated the good production of conidia. only TD1 isolate that had good relation between The growth and conidia production. This isolates had green colour full well than the others. *Trichoderma* isolate that had the green colour colony indicated that producted the dense conidia [8]. The best isolate as biological control agents must product the high conidia because the conidia is used as propagule for dissemination.

The Germination of Conidia.

Percentage of conidia germination of 15 *Trichoderma* spp isolates differed significantly (α 0,05). Generally conidia germination of *Trichoderma* spp indigenous various banana rhizosphere were categorized good, above 88,70%. There were 4 isolates had high conidia germination i. e : PP1, TD1, SO10 and TD3 with average conidia viability 97%-98,25%. (Table 4)

Table 4. Conidia Germination of *Trichoderma* spp that Incubated during 6 days at 28^{0}C

The Kind of Isolates	The Germination of Conidia (%)
PP 1	98.25 a
TD 1	97.50 a
S0 10	97.00 a
TD 3	97.00 a
TD 9	96.50 a b
PP 7	96.25 a b
SO 6	95.75 a b
SO 11	94.00 b c
TD 11	93.75 b c
SO 9	91.75 c d
SO 2	91.25 c d e
PP 4	91.25 c d e
PP 6	90.00 d e
TD 12	89.25 d e
TD 4	88.75 e
CV. 2.22%	

The figure 1 in the column followed by the same lowercase letter are not significantly different according to DNMRT at 5% level

All isolates that tested had good production of conidia. There were the relation between production of conidia with the conidia germination but not with the colony width. TD1, SO10, and TD3 that could product conidia highly also had the good conidia germination. Isolates with high conidia germination had big opportunity to cause antagonist mechanism. According to [9] the ability of *Trichoderma* to suppress the growth of pathogenic fungus was determined by conidia germination.

The viability of *Trichoderma* spp were different significantly among isolates (α 0,05). In this case caused by the different environment of native region of isolate that influence the growth and develop of *Trichoderma in vitro*. Estimating that 15 *Trichoderma* isolates from the centra production of banana in west Sumatera consisted of different species or strain so each isolate had different viability. According to Reference [7] *Trichoderma* spp from different subtrata and places in Poland had the height diversity of morphological and moleculer analysis.

The Effect of Temperature changes to Conidial Germanation

ISSN: 0975-8585

Treatment with various temperature influenced the germination of conidia significantly (α 0,05). At 20^{0}C and 25^{0}C germination of conidia of all isolates were high. All isolates were able to germinate above 90%. Increasing the temperature caused decrease conidia germination. (figure 1)

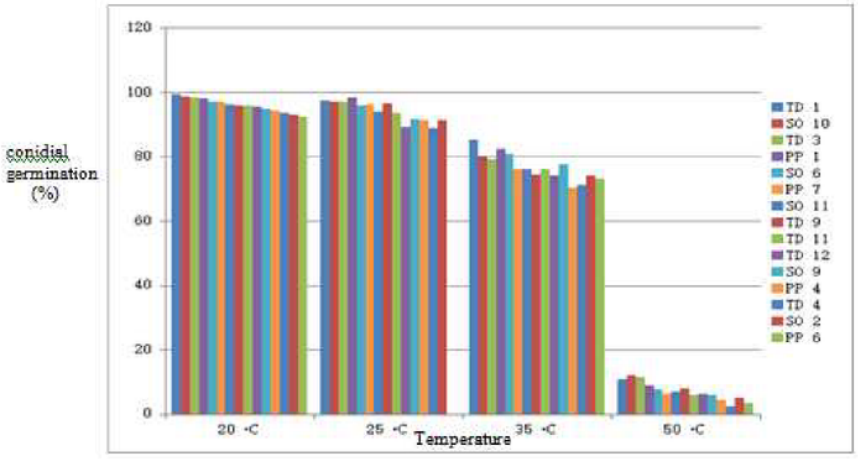

Figure 1: Effect of Temperature changes to Conidia Germination

PP1, TD1 and SO10 isolates were tolerant to high temperature treatment. Three isolates still had high conidia germination at 35^{0}C. [14] reported that conidia of *T. stromaticum* was able to germinate about 95% at temperature 20^{0}C-30^{0}C. The conidia germination of all isolates decreased at 35^{0}C and 50^{0}C. the reduction of conidia germination was various according to isolate and temperature used. Each isolate of *Trichoderma* spp gave different conidia germination response to temperature variation. At 35^{0}C the average of conidia germination was 70,25-87,25%. At 50^{0}C conidia germination of *Trichoderma* was very low, the highest conidia germination only 12%. This case was caused by at 50^{0}C is the above maximum temperature for germination of *Trichoderma* conidia. According to [14], the maximum temperature for conidia germination of *Trichoderma* spp were 37^{0}C.. PP1, TD1 and SO10 isolates were categorized as tolerant to high temperature treatment. The three isolates still high conidia germination after treated with several rates of temperature

Two isolates of *Trichoderma* had the best character were TD1 and SO10, those isolates had the good conidia production, conidia germination, and more tolerant to high temperature. The best character of those isolates had relation with ability to suppress Foc development, according to [11], TD1 and SO10 isolates had higher effectivity than other isolates in suppressing Foc infection in planta.

CONCLUSION

The viability of *Trichoderma* spp indigenus various banana rhizosphere in West Sumatera were different. There were two isolates that had better viability character and tolerant to high temperature than others. They are TD1 and SO10.

REFERENCES

[1] Samuel GJ. 1996. *Trichoderma*: a review of biology and systematic of the genus. Mycol.Res 100 (8): 923-935

[2] Druzhinina IS., Kopchinsky AG., Kubicek CP. 2006. The first 100 *Trichoderma* species characterized by molecular data. Mycoscience 47 (2): 55-64.

[3] Blaszczyk L., Delfina, P. Jerzy C., Grzegorz, K. Garys J.S.. Krzysztof and S. Marek 2011. Species Diversity of *Trichoderma* in Poland. Journal Applied Genetica 52 (2): 233-243

[4] G.E Harman.2000. Changes in Perception Derived from Research on *Trichoderma harzianum* T-22. *Plant Disease*. Publication No. D-2000-0208-01F.

[5] Hjeljord LG,. and Trosmo A. 2003. Effect of germination initiation on competitive capacity of *Trichoderma atroviride* P1 conidia. Phytopathology 93 (12);1593-1598.

[6] Kabir SE, Depnath S, Mazumder A, Dey T, Bera B. 2016. In vitro Evaluation of four Native *Trichoderma* spp Isolates Againts Tea Pathogens. Indian Journal of Fundamental and Applied Life Sciences (on line). 6 (2): 1 -6

[7] Nurbailis, Mardinus, Nasril N, Abdi D, Trimurti, H. 2006. Penapisan Isolat *Trichoderma* yang berasal dari Rizosfir Tanaman Pisang di Sumatera Barat untuk Pengendalian Penyakit Layu Fusarium. Jurnal Akta Agrosia. 9 (1) . 49 – 55.

[8] Gupta V, Sharma AK. 2013. Assesment of Optimum Temperature of *Trichoderma harzianum* by monitoring Radial Growth and Population Dynamic in Different Compost Manure and Different Temperatur. Octa Journal of Biosciences. 1 (2) 151-157.

[9] Poosapati S, Prasad DR, Navaneetha T, Dinesh KV, Sarad C. 2014. Selection of high Temperatureand Salinity Tolerant *Trichoderma* Isolates with Antagonistic Activity Againts *Sclerotium rolfsii*. Springerplus (3): 641,

[10] Daryaei A, Jones E, Ghazalibiglar EH. Glare TR, Falloon RE. 2016 Effect of Temperature, Light and Incubation Period on Production, Germination and Bioactivity of *Trichoderma atroviride.* Journal of Applied Microbiology, 120 (4): 999-1009

[11] Bae YS, Guy RK. 2000. Contransformation of *Trichoderma harzianum* with ß-Glucuronidase and Green Fluorescents Protein Genes Provides a Useful Tool for Monitoring Fungal Growth and Activity in Natural Soil. Applied and Enviromental Microbiology, 66 (2):.810-915.

JURNAL RJPBSC VOL 8 NO. 3 2017.pdf

ORIGINALITY REPORT

13%	5%	9%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	media.neliti.com Internet Source	3%
2	Submitted to Universitas Brawijaya Student Paper	2%
3	Lidia Błaszczyk. "Species diversity of Trichoderma in Poland", Journal of Applied Genetics, 04/05/2011 Publication	2%
4	Biological Management of Diseases of Crops, 2013. Publication	1%
5	repository.uinjkt.ac.id Internet Source	1%
6	Dal Bello, Gustavo, María Cristina Rollán, Gladys Lampugnani, Cecilia Abramoff, Lía Ronco, Silvina Larrán, Marina Stocco, and Cecilia Mónaco. "Biological control of leaf grey mould of greenhouse tomatoes caused by Botrytis cinerea", International Journal of Pest Management, 2011.	1%

Publication

7	Błaszczyk, Lidia; Siwulski, Marek; Sobieralski, Krzysztof; Lisiecka, Jolanta and Jędryczka, Małgorzata. "Trichoderma spp. -- application and prospects for use in organic farming and industry", Journal of Plant Protection Research, 2014. Publication	1%
8	f1000.com Internet Source	1%
9	"Physiological and Molecular Signalling Involved in Disease Management Through Trichoderma: An Effective Biocontrol Paradigm", Fungal Biology, 2016. Publication	1%
10	Daryaei, Amir, E. Eirian Jones, Hoda Ghazalibiglar, Travis R. Glare, and Richard E. Falloon. "Effects of temperature, light and incubation period on production, germination and bioactivity of Trichoderma atroviride", Journal of Applied Microbiology, 2016. Publication	<1%
11	L. Li. "Induction of Chlamydospores in Trichoderma harzianum and Gliocladium roseum by Antifungal Compounds Produced by Bacillus subtilis C2", Journal of Phytopathology, 12/2005	<1%

	Publication	
12	jom.unri.ac.id Internet Source	<1%
13	www.neliti.com Internet Source	<1%
14	P. Narayanasamy. "Biological Management of Diseases of Crops", Springer Nature, 2013 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		