Vol. 12(36), pp. 873-878, 28 September, 2018
DOI: 10.5897/AJMR2017.8721
Article Number: EFC5E5F58955
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Optimization of culture conditions for *Paecilomyces lilacinus* (Thom) Samson M-14

Xiaoqing Wei[1,2], Changzhong Liu[1] and Li Gao[2*]

[1]College of Plant Protection, Gansu Agricultural University, Lanzhou, China.
[2]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Beijing, China.

Received 27 September, 2017; Accepted 18 January, 2018

Paecilomyces lilacinus **is known as an effective parasite on nematodes which cause diseases to plants.** ***P. lilacinus*** **shows potential as a biocontrol agent against plant parasitic nematodes. The objective of this study is to optimize culture methods including nutritional requirements and environmental factors. The optimized culture conditions for biomass yields of** ***P. lilacinus*** **M-14 were spore suspension on basal medium (sucrose 19.00 g, soy peptone 4.06 g, K_2HPO_4 1.00 g, KCl 0.50 g, $MgSO_4$ 0.50 g, $FeSO_4$ 0.01 g and 17.00 g Bactor) for the first stage culture of 4 days under room condition for fungal growth, and then moved to another medium (maltose 5.00 g, soy peptone 2.50 g, $ZnSO_4 \cdot 7H_2O$ 0.25 gL^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 0.005 gL^{-1}, H_3BO_4 0.005 gL^{-1}, $CuSO_4 \cdot 5H_2O$ 0.01 gL^{-1} and 17.00 g Bactor) for another 4 days culture. The environmental factors combination was water potential -1.2 MPa/pH 3/light 12 h/temperature 29°C for biomass yields, and for sporulation of** ***P. lilacinus*** **M-14 under the environmental conditions, it was water potential -1.2 MPa/pH 3/24 h light/29°C. It will provide valuable insight into culturing of the biocontrol fungus.**

Key words: Biomass, environment, *Paecilomyces lilacinu,* biocontrol fungus.

INTRODUCTION

With the increase in awareness of the harmful effects of chemical pesticides and the changing public attitude towards environmental pollution, chemical pesticides is losing their popularity among farmers (Pandey et al., 2000; Anastasiadis et al., 2008). Environmental concerns for the quality of the environment and food safety have created social and legislative pressure to remove many agricultural pesticides from the market (Noling and Dickson, 1992; McKenry et al., 1994). Biological control is considered as the most safe and effective alternative tochemical control methods (Kutschera and Hossfeld, 2012; Sharma et al., 2014; Liu et al., 2017). Biocontrol agent, like *Paecilomyces lilacinus*, is a soil-inhabiting fungus that has shown great potential (Morgan-Jones et al., 1984; Jatala, 1986; Dube and Smart, 1987; Khan et al., 2006, Kepler et al., 2017; Chaverri et al., 2015; Yu et al., 2013), which has been reported to reduce *Meloidogyne incognita* (one of the most destructive pests of a wide range of crops, causing more than 10% loss in the world's total crop production) populations and this the

*Corresponding author. E-mail: lgao@ippcaas.cn.

world's total crop production) populations and this reduction was showed in the tomato yield (Lara et al.,1996; Topp et al., 1998). Mani et al. (1989) found that it has good biocontrol efficiency on many root-knot nematodes.

The aim of this study is to optimize the biomass yields of *P. lilacinus* based on its growth on a broad culture medium, such as PDA, PDB, PCA, Czapek and which have better growth and sporulation on Czapek, after 8 days culture, and has 1.10 g 50 mL^{-1} mycelia and 28.50×10^{6} mL^{-1} spore yields (Li et al., 2005). Suebsak (1996) found that the optimal culture medium was the juice of soy or potato, which contain more than 0.4 M $Mg^{2+}$and 0.1×10^{-3} M Cu^{2+} and have great suppression on the growth of this fungi. *P. lilacinus* could also grow on nature basis, including plant leaf, rice, wheat, and green pea (Mani et al., 1989; Abu-Laban and Saleh, 1992). Different nutrition leads to different mycelia and spore yields; it could produce more spores on rice than a green pea (Zaki and Bhatii, 1991; Xue et al., 2013). Siddiqui and Mahmood (1994) reported that the leaf extracts and residues of *Peristrophe bicalyculata* and *Dalbergia sissoo* were best as culture substrates in 17 plants for *P. lilacinus* in the fields.

Villanueva and Davide (1984) found that better mycelia growth was acid as compared to alkalescence, with a normal growth at 15-35°C, and a better growth and sporulation at 25-30°C. Suebsak found that the optimal culture medium was the juice of soy or potato under the temperature of 31°C with 220 to 270 r min^{-1} (Sun et al., 1997). Relative humidity is the key to germination of *P. lilacinus*, when RH reaches 85%, it began to germinate, with the highest germination at 98% RH under 25°C (Huang et al., 1994).

The combination effects of culture conditions, including nutrition and environmental factors on the growth and sporulation of *P. lilacinus* is reported in this study. This method is different from previous reports (Gao et al., 2009). This information will provide more details on the fungus' mass production.

MATERIALS AND METHODS

Fungal strain

The tested nematophagous fungus, *P. lilacinus* M-14 was originally isolated from *Heterodera glycines* from Heilongjiang (China), and deposited in the CGMCC in Institute of Microbiology, CAS.

Nutrition for the sporulation of *P. lilacinus* M-14

Yeast extract (Sigma Chemical Co.), maltose, $MgSO_4$, sucrose, starch soluble, $FeSO_4$, urea, K_2HPO_4, (Beijing Chemical Reagents Company, Beijing China), KCl (Nanjing Chemical Reagents Company, Nanjing China) and soy peptone (Shanghai Chemical Reagents Company, Shanghai China) were used in this study.

The basal medium included 17.00 g Bactor (Difco) agar, sucrose 19.00 g (equal to 8 g carbon), soy peptone 4.06 g (equal to 0.33 g nitrogen), K_2HPO_4 1.00 g, KCl 0.05 g, $MgSO_4$ 0.50 g, $FeSO_4$ 0.01 g per liter. This medium was used for the first stage culture for 4 days.

Effects of carbon concentrations and carbon to nitrogen ratios

Sucrose (42% carbon): 1, 2, 4, 8 and 16 gL^{-1} was used to adjust carbon concentrations, soy peptone (8% nitrogen): 0.2, 0.4, 0.8 and 1.6 gL^{-1} was used to adjust nitrogen concentrations, which resulted in C:N ratios ranging from 0.625:1 to 80:1. This was used for the second stage culture for sporulation for another 4 days. The optimal carbon concentration of 2 gL^{-1} with carbon to nitrogen ratio of 10:1 was obtained (Gao and Liu, 2009).

Effects of carbon and nitrogen sources combination

The combinations of sources include maltose, sucrose, starch soluble, soy peptone and yeast extract. Based on the carbon concentration, 2 gL^{-1} and C/N ratio of 10:1, the combinations of different carbon and nitrogen sources for sporulation with this novel method was obtained. For each combination, they were added to the basal medium to replace the sucrose and soy peptone as sporulation medium for the second stage culture of more 4 days. The basal medium for sporulation of another 4 days was used as a control.

Effects of mineral elements

After testing the components and concentration gradients of six mineral elements for sporulation of these two isolate with one-factor-at-a-time method, the optimal components for the sporulation of *P. lilacinus* M-14, including $ZnSO_4\cdot7H_2O$ 0.25 gL^{-1}, $Na_2MoO_4\cdot2H_2O$ 0.005 gL^{-1}, H_3BO_4 0.005 gL^{-1}, and $CuSO_4\cdot5H_2O$ 0.01 gL^{-1} was obtained.

Effects of environmental conditions on sporulation of *P. lilacinus* M-14 using the method

The two-stage cultivation method in the plates was used to evaluate the effects of pH, water potential, dark/light cycle and temperature on the second stage culture of 4 days more on sporulation of the biocontrol fungi. Water potential includes -0.3, -0.8, -1.2, -2.1,-3.9 and -7.3 MPa; pH includes 3, 4, 5, 6, 7, 8, 9, dark/light cycle includes 24/0 h, 12/12 h, 0/24 h, temperature includes 20, 23, 26, 29 and 32°C. In this study, two better levels of the orthogonal experiment were selected as shown in Table 1.

Optimization of the culture conditions

After the nutrition combination by full experiment, the combination of nutrition together with environmental factors was optimized for sporulation of *P. lilacinus* M-14 by $L_{16}(2^{15})$.

Statistical analysis

One-way analysis of variance (ANOVA) was used. Duncan's multiple range test was done using Statistical Analysis System (Version 8.2, SAS Institute, Cary, NC) to test the significant differences at $P = 0.05$.

RESULTS

The sources combination of carbon and nitrogen

The combination of carbon and nitrogen sources on

Table 1. Effects of environmental factors on sporulation of *P. lilacinus* M-14.

Factors	Water potential (MPa)	pH	Light (h)	Temperature (°C)
Level 1	-0.3	5	24	29
Level 2	-1.2	3	12	26

Table 2. Effect of carbon and nitrogen source on the sporulation of *P. lilacinus* M-14 (10^5 /ml).

Carbon sources	Nitrogen sources		CK	LSD
	Yeast extract	Soy peptone		
Starch soluble	72.8^{d}	79.2^{d}		
Maltose	39.7^{e}	319.3^{a}	45.0^{e}	12.21
Sucrose	142.5^{b}	126.0^{c}		

sporulation of the isolates showed significant effects (Table 2). The combination of maltose and soy peptone showed the best sporulation.

Optimization of the conditions

According to the four factors and two levels shown in Table 1, $L_{16}(2^{15})$ was used to optimize the experimental conditions (Table 3). Based on the orthogonal method, the results are showed in Table 3, and the order of effects of all factors on mycelia growth could be determined as 32.12 (water potential) > 16.62 (pH) > 6.71 (light) > 4.29 (temperature) according to R (maximum difference) in Table 4.

ANOVA results showed that the water potential had significant effects on biomass yields and pH had significant effects on sporulation (Table 5). The effect of combinations of four factors on biomass yields and sporulation is shown in Table 6. The combinations of B2/A2, A1/C2, B2/C2, A1/D2, D1/B2 and D2/C2 could produce more biomass yields (176.25, 173.17, 165.67, 172.67, 169.75 and 161.75 (mg per colony), respectively). The optimum factors for high mycelia yields are water potential -1.2MPa (A2)/pH 3 (B2)/12 h light (C2)/29°C (D2) (Table 4). The optimum factors for high spore yields are water potential- 1.2 MPa (A2)/pH 3 (B2) /24 h (C1) light /29°C (D2).

DISCUSSION

Cultivation with two-stage method

The method was used to optimize the biomass and sporulation of nematophagous fungus separately. With the help of membranes of cellophane, the basal medium for first stage of 4 days for fungal growth was transferred to the second stage of 4 days sporulation culture. The nutrition and two better levels of 4 environmental factors on sporulation of *P. lilacinus* M-14 were then combined by orthogonal matrix method to obtain better combinations.

Effects of carbon and nitrogen sources

Some nutritional components could accelerate the sporulation of *P. lilacinus* M-14, while their combination may not be the best for its sporulation. These results proved this phenomenon, which also indicated that the full experiment of nutrition was necessary, and also the orthogonal method was essential for the sporulation of *P. lilacinus* M-14 on nutrition and environmental factors.

Optimization by orthogonal matrix method

Based on the results, the research combined the nutritional components environmental factors, which is different from other reports which only referred to one fields. In this study, water potential played an important role in biomass, while pH was the key to sporulation.

Combinations of the three fields

The nutritional components can significantly influence growth and sporulation of many fungi (Culbreath et al., 1986; Tiganomilani et al., 1995; Rao et al., 1997), which showed the essential to optimize nutrition for the fungus. The nutrition for the fungal biomass and sporulation may not necessarily correlate under the orthogonal matrix method, which also indicated the essential of two-stage method.

Basis for formulation and storage conditions

Instability of the biofungicide greatly limited their

Table 3. Orthogonal experiment of $L_{16}(2^{15})$ of biomass yields and sporulation of *P. lilacinus* M-14.

Exp. group	A	B	A×B*	C	A×C	B×C		D	A×D	B×D		C×D				Biomass yields (mg per colony)	Sporulation (10^5 per colony)
1***	1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	147.33 ± 36.02****	2.79 ± 0.47
2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	173.67 ± 6.35	3.06 ± 0.01
3	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	176.33 ± 5.03	2.80 ± 0.21
4	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	174.33 ± 1.53	2.09 ± 0.04
5	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	164.00 ± 6.08	2.89 ± 0.20
6	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	192.33 ± 2.89	3.21 ± 0.06
7	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	182.67 ± 7.64	3.16 ± 0.07
8	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	162.00 ± 18.08	3.11 ± 0.03
9	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	165.33 ± 6.66	2.40 ± 0.07
10	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	176.67 ± 4.73	2.74 ± 0.03
11	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	149.33 ± 4.04	2.93 ± 0.06
12	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	151.67 ± 9.07	2.48 ± 0.06
13	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	110.33 ± 34.50	2.85 ± 0.23
14	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	91.67 ± 5.51	2.91 ± 0.07
15	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	131.67 ± 31.50	2.82 ± 0.05
16	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	143.00 ± 9.85	2.87 ± 0.02

*A×B, A×C, B×C, A×D, B×D and C×D indicates the interactions between the factors: water potential and pH, water potential and light, pH and light, water potential and temperature, pH and temperature, light and temperature, respectively. ** The columns were categorized as orthogonal design for $L_{16}(2^{15})$. ***Every row of the experimental group number represents one experimental replicate, and every experimental group was replicated thrice. **** Values are mean ± SD of triple determinations.

Table 4. Analysis of environmental factors on biomass production and sporulation of *P. lilacinus* M-14 with this novel method.

		A	B	A×B	C	A×C	B×C		D	A×D	B×D		C×D			
B*	K_1	1376.66	1314.66	1148.33	1221.33	1253.00	1286.34	1172.67	1230.99	1237.34	1227.32	1248.33	1217.99	1197.00	1241.00	1279.99
	K_2	1119.67	1181.67	1348.00	1275.00	1243.33	1209.99	1323.66	1265.34	1258.99	1269.01	1248.00	1278.34	1299.33	1255.33	1216.34
	k_1	172.08	164.33	143.54	152.67	156.63	160.79	146.58	153.87	154.67	153.42	156.04	152.25	149.63	155.13	160.00
	k_2	139.96	147.71	168.50	159.38	155.42	151.25	165.46	158.17	157.37	158.63	156.00	159.79	162.42	156.92	152.04
	R	32.12	16.62	24.96	6.71	1.21	9.54	18.87	4.29	2.71	5.21	0.04	7.54	12.79	1.79	7.96
	O	1	1	2	2	1	1	2	2	2	2	1	2	2	2	1
S†	K_1'	23.19	21.29	22.19	22.85	23.05	22.95	23.29	22.64	23.02	22.80	21.48	21.48	22.28	21.86	22.64
	K_2'	22.00	23.82	22.92	22.26	22.06	22.16	21.82	22.47	22.09	22.31	23.63	23.63	22.83	23.28	22.47
	k_1'	2.90	2.66	2.77	2.86	2.88	2.87	2.91	2.83	2.88	2.85	2.69	2.69	2.79	2.73	2.83
	k_2'	2.75	2.98	2.87	2.78	2.76	2.77	2.73	2.81	2.76	2.79	2.95	2.95	2.85	2.91	2.81
	R'	0.14	0.32	0.09	0.07	0.12	0.10	0.18	0.02	0.12	0.06	0.27	0.27	0.07	0.17	0.02
	O'	1	2	2	1	1	1	1	1	1	1	2	2	2	2	1

*Biomass yields (mg per colony). † Sporulation (105 conidia per colony). K1 and K2 are the total content of biomass yields from the level 1 and level 2 separately; k1 and k2 are the mean value of levels 1 and 2 separately.K1' and K2' are the total spore yields from the level 1 and level 2 separately; k1' and k2' are the mean value of levels 1 and 2 separately. R is the maximum of k1, k2 minus the minimum of k1, k2 and R' is the maximum of k1, k2 minus the minimum of k1, k2 respectively. O is the optimal level of biomass yields and O' is the optimal value of spore yields.

Table 5. The variance analysis of $L_{16}(2^{15})$ orthogonal test on optimation of environmental factors for biomass yields and sporulation of *P. lilacinus* M-14.

Parameter	Variance source	Sum of square deviation (SS)	Degree of freedom (v)	Mean square (MS)	*F*-ratio	Significance level†
Biomass yields (mg per colony)	A	4127.74	1	8555.78	8.80	*
	B	1105.40	1	1764.21	2.36	
	C	180.03	1	1.00	0.38	
	D	73.75	1	1024.16	0.16	
	A×B	3738.35	1	3738.35	1.59	
	A×C	1252.39	1	1252.39	0.53	
	A×D	1275.85	1	1275.85	0.54	
	B×C	1610.82	1	1610.82	0.69	
	B×D	1355.16	1	1355.16	0.58	
	C×D	1476.68	1	1476.68	0.63	
	Error	2345.49	5			
Sporulation (10^5 conidia per colony)	A	0.08	1	0.22	1.32	
	B	0.40	1	0.01	6.85	*
	C	0.022	1	0.002	0.38	
	D	0.01	1	0.01	0.01	
	A×B	0.044	1	0.044	0.15	
	A×C	0.072	1	0.072	0.25	
	A×D	0.07	1	0.07	0.23	
	B×C	0.05	1	0.05	0.17	
	B×D	0.026	1	0.026	0.09	
	C×D	0.30	1	0.30	1.04	
	Error	0.290	5			

†F0.1 (1,5) = 4.06, F0.05 (1,5) = 6.610, F0.01 (1,5) = 16.3. *F-ratio >F 0.1. **F 0.1 < F-ratio < F0.05. *** F-ratio < F0.01.

Table 6. Effects of combinations of environmental factors on biomass yields and sporulation of *P. lilacinus* M-14.

B, C or D	A				B				C			
	A_1		A_2		B_1		B_2		C_1		C_2	
	B†	S‡	B	S	B	S	B	S	B	S	B	S
B_1	167.92	2.69	119.17	2.86								
B_2	160.75	2.64	176.25	3.09								
C_1	155.50	2.82	157.75	2.95	148.92	2.89	164.33	2.88				
C_2	173.17	2.51	137.67	3.01	138.17	2.66	172.67	2.85				
D_1	163.00	2.70	146.34	2.96	139.58	2.84	169.75	2.82	151.50	2.76	157.84	2.90
D_2	165.67	2.62	149.08	3.00	147.50	2.71	167.25	2.91	161.75	2.61	153.00	3.01

A1, A2, B1, B2, C1, C2, D1, D2 represent the 1 and 2 levels of water potential, pH, light and temperature. † Represent the biomass yields (mg per colony). ‡ Represent spore yields (105 conidia per colony).

application, with better nutrition and environmental storage conditions helping in their resistant to unfavorable conditions (Miller et al., 1997). These results could also help in selecting better material to formulate for a longer shelf life (for example, some fungi are sensitive to light, and the material resistant to light can be chosen). In addition, chitin helps in stabilizing the fungi and also for a better activity in microbiology with a better control efficiency in pests (Rodriguez et al., 1984; Sun et al., 1997).

Conclusion

In summary, the culture conditions for biomass yields and sporulation of *P. lilacinus* M-14, were optimized, which will provide valuable information on mass production (both yields of biomass and spore) of the potential biocontrol agent.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by Beijing Natural Science Foundation (L140012), Multidisciplinary Cooperation Project of Beijing Nova Program (Z14111000180000) and Beijing Nova Program (Z131105000413057).

REFERENCES

Abu-Laban AZ, Saleh HM (1992). Evaluation of animal manures for mass production, storage and application of some nematode eggs parasitic fungi. Nematologica 38(1):237-244.

Anastasiadis IA, Giannakou IO, Prophetou-Athanasiadou DA, Gowen SR (2008). The combined effect of the application of a biocontrol agent paecilomyces lilacinus, with various practices for the control of root-knot nematodes. Crop Protection 27(3):352-361.

Culbreath AK, Rodriguez-Kabana R, Morgan-Jones G (1986). Chitin and *Paecilomyces lilacinus* for control of *Meloidogyne arenaria*. Nematropica 16:153-166.

Dube B, Smart GC (1987). Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19(2):222-227.

Gao L, Liu XZ (2009). A novel two-stage cultivation method to optimize carbon concentration and carbon-to-nitrogen ratio for sporulation of biocontrol fungi. Folia Microbiologica 54(2):142-146.

Gao L, Liu XZ, Sun MH, Li SD, Wang JL (2009). Use of a novel two-stage cultivation method to determine the effects of environmental factors on the growth and sporulation of several biocontrol fungi. Mycoscience 50(4):317-321.

Huang JC, Li F, Chen JJ (1994). Effect of some enviramental factors on the condial germination and growth of *Paecilomyces lilacinus*. Journal of Fujian Agriculture and Forestry University 23(3):298-302.

Jatala P (1986). Biological control of plant parasitic nematodes. Annual Review of Phytopathology 24:453-489.

Khan A, Williams KL, Nevalainen HKM (2006). Control of plant parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials. Biocontrol 51(5):643-658.

Kutschera U, Hossfeld U (2012). Physiological phytopathology: origin and evolution of a scientific discipline. Journal of Applied Botany and Food Quality 85(1):1-5.

Lara J, Acosta N, Betancourt C, Vincente N, Rodriguez R (1996). Biological control of *Meloidogyne incognita* in tomato in puerto rico. Nematropica 26(2):143-152.

Li F, Huang SF, Liu B (2005). Growing characteristics of *Paecilomyces lilacinus* (Thom.) Samson NH2PL203 on different media. Plant Protection 31(3):46-49.

Mani A, Murthy IR, Rao PK (1989). Growth of *Paecilomyces lilacinuson* natural substrates and its efficacy against citrus nematode, *Tylenchulus semipenetrans.* Journal of Biological Control 3(1):59-61.

McKenry M, Buzo T, Kretsch J, Kalu S, Otomo E, Ashcroft R, Lange A, Kelley K (1994). Soil fumigants provide multiple benefits: alternatives give mixed results. California Agriculture 48(3):22-28.

Miller RJ, Baker GL, Hooper GHS, Prior C (1997). Development of a mycoinsecticide for the Australian plague locust. In. Krall S, Eveling R, Ba Diallo (eds.), New Strategies in Locust Control Birkhauser Verlag. Basle. pp. 177-183.

Morgan-Jones G, White GF, Rodriguez-Kabana R (1984) .Phytonematode Pathology: Ultrastructural Studies. II. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. Nematropica 14(1):57-71.

Noling JW, Dickson DW (1992). The face of methyl bromide within florida agriculture. Citrus and Vegetable Magazine pp.19-24.

Pandey R, Kalra A, Tandon S, Mehrotra N, Singh HN, Kumar S (2000). Essential oils as potent sources of nematidical compounds. Journal of Phytopathology 148(7):501-502.

Rao MS, Reddy PP, Nagesh M (1997). Integrated management of *Meloidogyne incognita* on okra by castor cake suspension and *Paecilomyces lilacinus*. Nematologia Mediterranea 25(1):17-19.

Rodriguez KR, Moegan JG, Godoy G, Gintis BO (1984). Effectiveness of species of *Gliocladium*, *Paecilomyces* and *Verticillium* for control of *Meloidogyne arenaria* in field soil. Nematropica 14(2):10-25.

Sharma A, Sharma S, Yadav S, Naik SN (2014). Role of Karanja deoiled cake based medium in production of protease and fatty acids by *Paecilomyces lilacinus* 6029. Journal of Bioscience and Bioengineering 118(3):270-271.

Siddiqui ZA, Mahmood I (1994). Culture of *Paecilomyces lilacinus* on leaf extracts and leaf residues for nematode control. Bioresource Technology 49(2):187-189.

Suebsak S (1996). Production factors of *Paecilomyces lilacinus* a nematode parasite fungus in culture broth Kasetsart. Journal of Nature and Science 30:13-26.

Sun MH, Liu XZ, Tang L (1997). Fungistatic effect of soils on nematophagous fungi and their preparations. Mycosystema 16(2):149-154.

Tiganomilani MS, Carneiro RG, Defaria MR, Frazao HS, McCoy CW (1995). Isozyme characterization and pathogenicity of *Paecilomyces fumosoroseus* and *P. lilacinus* to *Diabrotica speciosa* (Coleoptera: Chrysomelidae) and *Meloidogynejavanica* (Nematode:Tylenchidae). Biological Control 5(3):378-382.

Topp E, Miller S, Bork H, Welsh M (1998). Effects of marigold (Tagetes sp.) roots on soil microorganisms. Biol. Fertil. Soils 27(2):149-154.

Villanueva LM, Davide RG (1984). Influence of pH, temperature, light and agar media on the growth and sporulation of a nematophagous fungus, *Paecilomyces lilacinus*. Philippine Agricultural Scientist 67:228-231.

Zaki FA, Bhatii DS (1991). Effect of culture media on sporulation of and its efficacy against *Medloidogy javanica* in tomato. Nematologia Mediterranea 19(2):211-212.