Journal of Science and Technology ***55*** *(1A) (2017) 45-53*
DOI: 10.15625/2525-2518/55/1A/12379

SELECTION OF INDIGENOUS STRAINS (*Paecilomyces lilacinus)* PARASITIZE *Meloidogyne* spp. ISOLATED FROM BA RIA – VUNG TAU PROVINCE

Nguyen Thi Thuy Duong[1, *], Vo Thi Thu Oanh[2], Duong Hoa Xo[1]

[1]*Biotechnology Center of Ho Chi Minh City, 2374 Highway 1, District 12, Ho Chi Minh City*
[2] *Nong Lam University, Block 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City*

[*]Email: *nguyenduongbiology@gmail.com*

Received: 30 October 2016; Accepted for publication: 30 May 2017

ABSTRACT

Twenty two *Paecilomyces lilacinus* strains were isolated from forest soils and black pepper rhizospheres in Ba Ria –Vung Tau Province. The ability to degrade chitin of PB 3.3, PB 2.9, QT2, and QT5 strains was high. The ability to degrade casein of PB 1.3, PB 2.10, KL5, and KL6 strains was efficient. And then, these strains were parasitized females and egg masses of *Meloidogyne* spp. *in vitro*. In female parasitism test, the rates of parasitizing female nematodes reached more than 50 % after treating for 2 days. Four strains of PB 2.10, PB 1.3, KL6 and QT5 belonged to the first group achieved the highest parasitic (> 90 %) effects on female after 3 days of incubation. In egg masses parasitism test, three strains of PB 1.3, PB 2.10 and QT5 exhibited 83.33 %, 75 % and 75 % parasitism on egg masses after 11 days of incubation. The rates of parasitizing female were higher than egg masses. Three selected strains from the experiments were PB 1.3, PB 2.10 and QT5.

Keywords: *Paecilomyces lilacinus*, chitinase, protease, parasitize.

1. INTRODUCTION

Meloidogyne spp., root-knot nematodes, are serious pests of many crops. First, these nematodes infect the roots of plants. The created wounds on root surface are gates of infecting of soil fungi such as *Fusarium* spp., *Phytophthora* spp., *Sclerotium rolfsii*, and *Rhizoctonia solani*. It significantly reduces the yield of black pepper in Ba Ria-Vung Tau Province and other areas in Vietnam [1]. *Paecilomyces lilacinus* is a common saprobic fungus. It grows fast and has less influence by the antagonistic fungi, and plant pathogenic fungi [2]. *Paecilomyces lilacinus* is capable of infecting eggs and females of the root-knot nematode *Meloidogyne* spp. [3, 4]. Infecting process of fungi was based on secreting extracellular protease and chitinase [5, 6]. The aims of this study were to test the ability of *Paecilomyces lilacinus* strains (isolated from the coastal forests of Binh Chau – Phuoc Buu and black pepper rhizospheres in Ba Ria-Vung Tau Province) to secrete extracellular enzymes and parasitizing on egg masses and female nematodes (*Meloidogyne* spp.) *in vitro*.

2. MATERIAL AND METHODS

2.1. Material

Table 1. List of *Paecilomyces lilacinus* strains.

Name of strains	Sampling locations	Soil pH
PB 1.1	IIIA2 state, Binh Chau -Phuoc Buu Forest, Xuyen Moc district	6.8
PB 1.3		6.8
PB 1.7		6.8
PB 1.10		6.8
PB 2.9	IIB state, Binh Chau -Phuoc Buu Forest, Xuyen Moc district	7.0
PB 2.10		7.0
PB 3.1	Suoi Can, Binh Chau -Phuoc Buu Forest, Xuyen Moc district	6.0
PB 3.3		6.0
PB 3.4		6.0
KL1	Kim Long commune, Chau Duc district	5.3
KL3		5.2
KL4		5.6
KL5		4.8
KL6		5.0
KL8		5.0
QT1	Quang Thanh commune, Chau Duc district	6.2
QT2		6.6
QT3		6.2
QT4		5.9
QT5		6.6
QT6		6.0
NG2	Ngai Giao town, Chau Duc district	6.2

Twenty two strains of *Paecilomyces lilacinus* were isolated from forest soils and black pepper rhizospheres in Ba Ria –Vung Tau Province (Table 1) and stored in the collection of

microorganism of Biotechnology Center of Ho Chi Minh City. Egg masses and females of *Meloidogyne* spp., which was cultured in greenhouse were splitted the galls from root black pepper.

2.2. Methods

2.2.1. Qualitative test of extracellular enzymes

Isolated fungi were cultured on PDA medium for 5 days. The fungal blocks (ca. 5 mm-diameter) was cut from the margin of the colony and transferred up-side-down Petri plates (ca. 90 mm-diameter) containing 9 ml medium (4.56 g K_2HPO_4, 2.77 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$ and 0.5 g KC1, 16 g agar, pH = 6.0) [7], and 1 % casein (be suspended in phosphate buffer [8]) for testing protease or 1 % chitin (be suspended in concentrated HCl [9]) for testing chitinase. The experiment was arranged in Randomized Complete Block Design (RCBD) with three times for each strain.

Observation: colony diameter (d, mm), formation of clearing zones around the colony diameter (D, mm) after 24, 48 and 72 hours of incubation.

2.2.2 Pathogenicity test on egg masses and female nematodes in vitro

Egg masses and female nematodes were collected by the method of Hussey and Barker [10] with some modifications. Roots with galls were washed in running tap water for 3–5 minute to remove soil. The females and egg masses were sterilized with 1 % sodium hypochlorite (NaOCl) for 1 min. And then they were washed with sterile distilled water in three times [11].

Selected strains (*Paecilomyces lilacinus)* with good extracellular enzymes were cultured on PDA medium from 5 to 7 days. Fungal blocks (5 mm-diameter) were excised, and then they were transferred to Petri plates (90 mm-diameter) containing 1.5 % water agar medium (WA). Three fungal blocks were placed in a group as three replications per a Petri plate for observing. Petri dishes were incubated for 5 days at 25 ^oC for mycelial growth. Eight egg masses or females were placed on the surface of each fungal piece and were cultured at 25 ^oC. They were directly observed under a stereomicroscope to count number of fungi-invading egg masses or females at 1, 2, 3, 4, 5, 7, 8, 9, 10 and 11 days after treatment [12]. The experiment was arranged in Randomized Complete Block Design (RCBD).

With a large number of condiophores radiating from the egg mass or female body, it was parasitized afer recording the rate of parasitized egg masses or females.

2.3 Data analysis

Data were statistically analyzed with ANOVA at P = 0.05 by SAS 9.1. The treatment mean values were compared with Duncan's new multiple range test (DNMRT).

3. RESULTS AND DISCUSSION

3.1. Result in qualitative test of extracellular enzymes

Table 2. The ability to degrade chitin and casein of *Paecilomyces lilacinus* strains isolated from Binh Chau Phuoc Buu forest.

Isolated strains	The ability to degrade chitin D-d (mm)				The ability to degrade casein D-d (mm)			
	24h	48h	72h	Mean	24h	48h	72h	Mean
PB 1.1	2.47	5.35abc	8.42b	5.41	6.20bcd	9.98a	7.87ced	8.02
PB 1.3	1.95	4.61bc	3.72cd	3.43	8.77a	8.68a	9.4b	8.95
PB 1.7	2.86	4.01c	3.93c	3.60	6.82abc	6.67b	6.45e	6.65
PB 1.10	2.17	4.26bc	2.94cd	3.13	8.79a	9.45a	6.84ed	8.36
PB 2.9	2.68	5.68ab	9.70b	6.02	4.72dc	5.29b	7.81ced	5.94
PB 2.10	2.04	4.5bc	2.88cd	3.14	7.15ab	10.58a	11.14a	9.63
PB 3.1	2.02	4.05c	1.22d	2.43	7.24ab	8.84a	8.67bc	8.26
PB 3.3	2.53	6.55a	13.95a	7.68	4.13d	5.76b	8.63bc	6.18
PB 3.4	2.49	4.32bc	3.06cd	3.29	4.51d	6.39b	8.21bcd	6.37
CV (%)	37	17	24		18	13	9	

The treatment means were separated according to Duncan's new multiple range test (DNMRT). Means in the same column followed by the same letter are not significantly different (P= 0.05).

Protease and chitinase were secreted by *Paecilomyces lilacinus* to degrade the nematode eggshell and cuticle of female. Table 2 was recorded the level of extracellular enzyme (D-d (mm)) of *Paecilomyces lilacinus* strains obtaining from forest soils Binh Chau Phuoc Buu after 24 hours, 48 hours, and 72 hours of incubation. The strain was capable of secreting high chitinase, but the ability to secrete protease decreased and vice versa. After 24 h of cultivation, the ability to secrete chitinase of isolated strains from forest land had no statistically significant difference. At the time of 48 hours and 72 hours after incubation, the ability to secrete chitinase of these strains had statistically significant difference. Two strains (PB 3.3 and PB 2.9) were the best activity on degrading chitin.

With the level of secreting extracellular protease, clearing zones of strains had statistically significant difference after 24h, 48h and 72h of incubation. At the time of 24 hours cultivation, PB1.3 strain and PB 1.10 strain had ability to secrete protease highly; moreover, after 48 hours and 72 hours, PB 1.3 strain and PB 2.10 strain had ability to secrete protease greatly (Figure 1). In this study, the ability to degrade chitin of PB 3.3 and PB 2.9 were the highest while PB 2.10 and PB 1.3 could secrete efficiently protease.

Table 3 was recorded the secreted level of extracellular enzyme (D-d (mm)) of *Paecilomyces lilacinus* strains obtaining from black pepper rhizospheres (Chau Duc District) after 24 hours, 48 hours and 72 hours of incubation. Two strains of secreting high chitinase were QT5 (Figure 2) and QT2 and the high level of secreting extracellular protease was KL5 and KL6. According to this experiment, isolated strains from Ba Ria-Vung Tau Province were able to secrete extracellular protease and chitinase. We chose 8 effective strains per 22 strains such as PB 1.3, PB 2.9, PB 2.10, PB 3.3, QT2, QT5, KL5 and KL6. These strains will be used for parastizing female and egg masses nematodes *in vitro*.

Table 3. The ability to degrade chitin and casein of *Paecilomyces lilacinus* strains isolated from black pepper rhizospheres in different time.

Isolated Strains	The ability to degrade chitin D-d (mm)				The ability to degrade casein D-d (mm)			
	24h	48h	72h	Mean	24h	48h	72h	Mean
KL1	2.53fg	5.29bc	5.52ef	4.45	6.94bc	8.45abc	9.10bcde	8.17
KL3	3.59bc	6.09abc	4.07f	4.59	6.64bc	7.21bcd	9.94bcd	7.94
KL4	2.55efg	4.59c	10.07cd	5.74	6.88bc	6.29cde	9.14bcde	7.44
KL5	3.87b	6.90abc	12.95c	7.91	7.48bc	10.17a	12.51a	10.06
KL6	2.67efg	6.35abc	4.25f	4.40	9.95a	8.64ab	11.30ab	9.97
KL8	2.67defg	5.01bc	6.16ef	4.62	6.73bc	5.48de	8.72bcde	6.98
QT1	3.33bcd	8.21a	7.02def	6.19	7.99b	9.98a	8.18de	8.72
QT2	2.84defg	6.97abc	17.8b	9.21	6.19c	7.49bcd	8.38cde	7.36
QT3	3.37bcd	7.18ab	5.56ef	5.37	7.50bc	10.25a	7.75de	8.51
QT4	3.26bcde	6.30abc	6.80def	5.46	7.07bc	7.46bcd	7.26de	7.27
QT5	6.33a	8.19a	28.54a	14.34	6.71bc	4.85e	6.52e	6.03
QT6	3.02cdef	7.37ab	8.69de	6.37	6.92bc	9.18ab	8.43cde	8.18
NG2	2.26g	6.73abc	7.63def	5.54	6.34bc	7.67bcd	10.93abc	8.32
CV (%)	11	19	20		12	15	15	

The treatment means were separated according to Duncan's new multiple range test (DNMRT). Means in the same column followed by the same letter are not significantly different (P= 0.05).

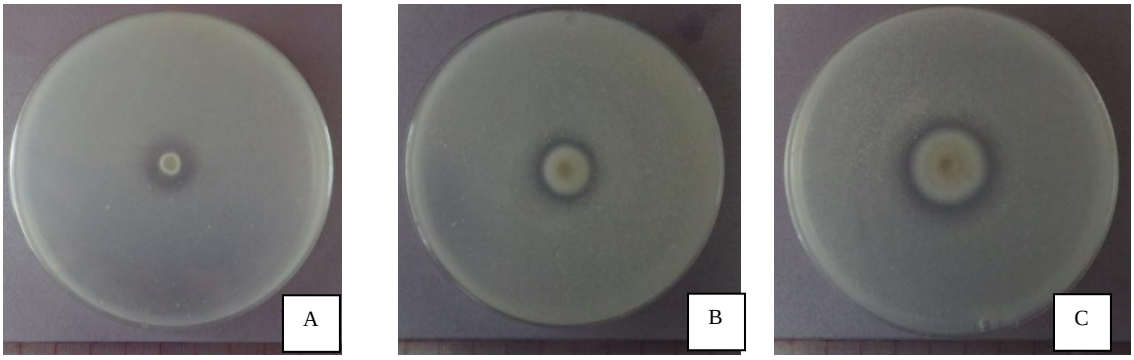

Figure 1. The clearing zones around the colony of PB2 10, after 24 hours (A), 48 hours (B), 72 hours (C) of incubation on agar plate supplement with casein.

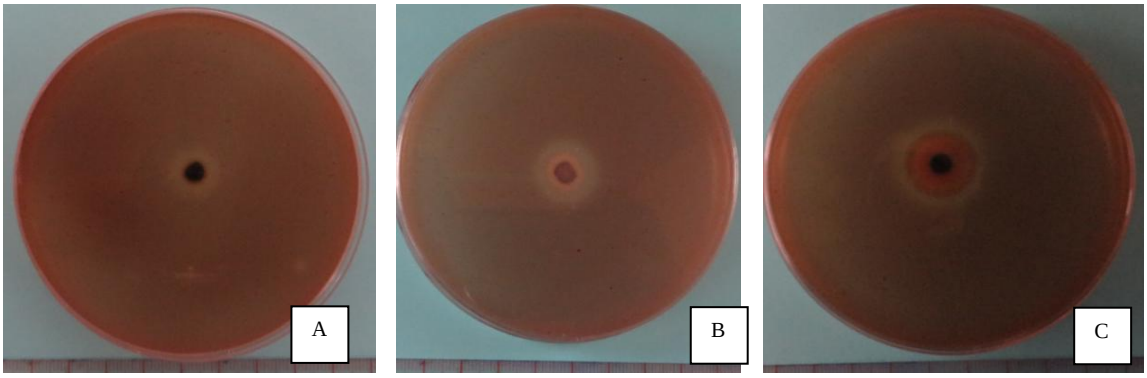

Figure 2. The clearing zones around the colonies of QT5, after 24 hours (A), 48 hours (B), 72 hours (C) of incubation on agar plate supplement with chitin.

3.2 Result in pathogenicity test on egg masses and female nematodes *in vitro*

Table 4. The rates of female nematodes were parasitized by *Paecilomyces lilacinus* strains *in vitro*.

Strains	Days after inoculation			
	1 day	2days	3days	4days
PB 1.3	8,33	79.17a	87.50ab	91.67ab
PB 2.9	0	58.33b	75.00bc	83.33ab
PB 2.10	0	83.50a	95.83a	95.83a
PB 3.3	0	75.00a	83.33ab	83.33ab
KL5	0	75.67a	83.33ab	87.50ab
KL6	0	83.33a	87.50ab	93.50ab
QT2	0	41.67c	66.67c	75.00b
QT5	0	83.33a	95.83a	95.83a
CV (%)	99	12.89	9.96	13.16
$F_{tính}$	1.00^{ns}	5.99	3.48	1.16

The treatment means were separated according to Duncan's new multiple range test (DNMRT). Means in the same column followed by the same letter are not significantly different (P = 0.05).

The rates of parasitizing female *Meloidogyne* spp. nematode parasitized rate after 1 day was changed $\overline{x + 0.5}$ before the analyzed statistic.

The rates of parasitizing female nematodes of 8 strains were reported in Table 4. At 2 days after treating, QT2 strain reached 41.67 % parasitism on female. After treating for 4 days, the rates of parasitizing female reached over 75 %. Two strains of PB 2.10 and QT5 had the rates of parasitizing female exhibited the highest (Figure 3).

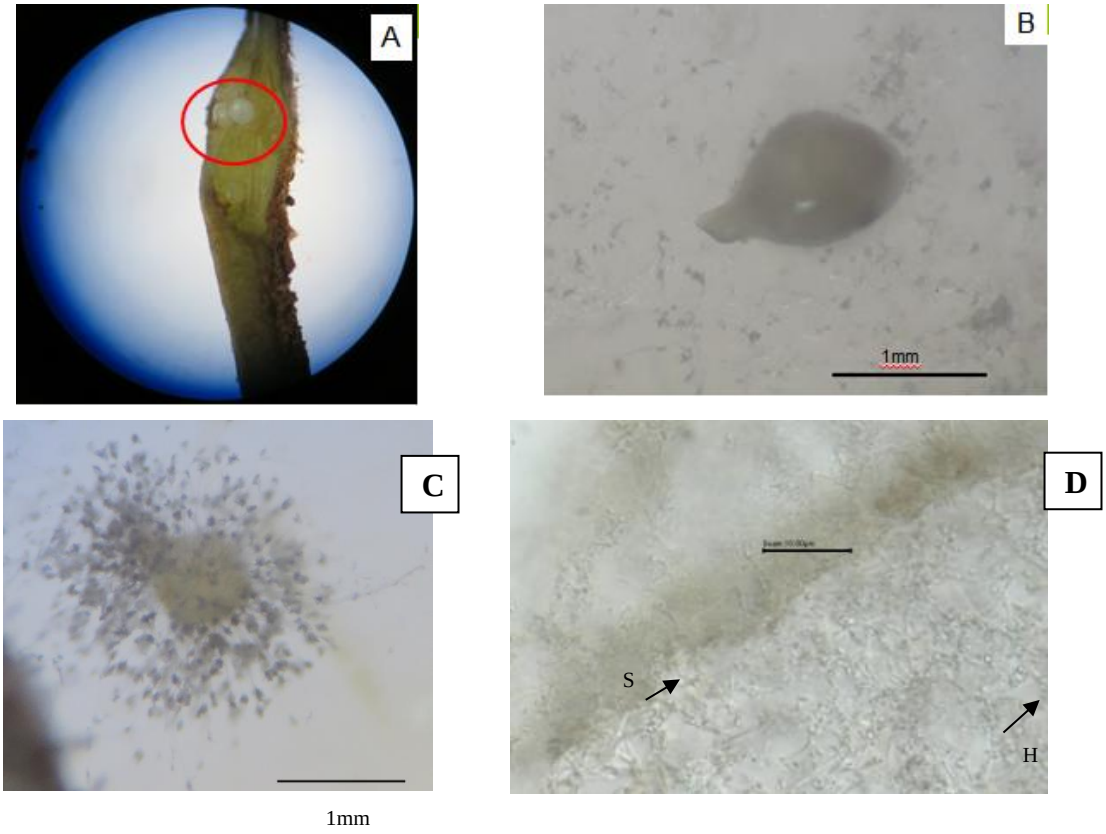

Figure 3. (A) Female of *Meloidogyne* sp., (B) Before the female was parasitized, (C) After the female was highly infected with *P. lilacinus* with a large number of condiophores radiating from the female body. The photographs were taken using stereomicroscope, the bar represents 1 mm. (D) Sample sliced sample of parasitized female of *Meloidogyne* sp. was observed under the microscope (40X). *S = Femal nematode shell, H = fungal hyphae.* Scale bar = 10 µm.

Table 5. The rates of parasitizing female at *invitro.*

Strains	Days after inoculation								
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
PB 1.3	33.33a	41.66a	45.83ab	54.16a	58.33a	66.66a	66.66a	75a	83.33a
PB 2.9	8.33b	8.33b	8.33d	8.33c	12.5c	20.83b	25b	33.33b	41.66bc
PB 2.10	16.66ab	25ab	29.16abc	37.5ab	41.66ab	45.83ab	58.33a	75a	75ab
PB 3.3	12.5ab	16.66ab	16.66bdc	20.83bc	20.83bc	29.16b	33.33b	37.5ab	37.5bc
KL5	4.16b	8.33b	8.33d	8.33c	12.5c	20.83b	20.83b	20.83b	25c
KL6	8.33b	8.33b	12.5cd	16.66bc	20.83bc	25b	29.16b	41.66ab	41.66bc
QT2	12.5ab	12.5ab	12.5cd	12.5bc	16.66bc	20.83b	25b	37.5ab	37.5bc
QT5	33.33a	37.5a	50a	50a	58.33a	66.66a	70.83a	75a	75ab
CV (%)	69	40	31	30	30	20	17	20	19

The treatment means were separated according to Duncan's new multiple range test (DNMRT). Means in the same column followed by the same letter are not significantly different (P= 0.05).

Egg masses Meloidogyne spp. *nematode rate were changed* $\overline{x + 0.5}$ *before the analyzed statistic.*

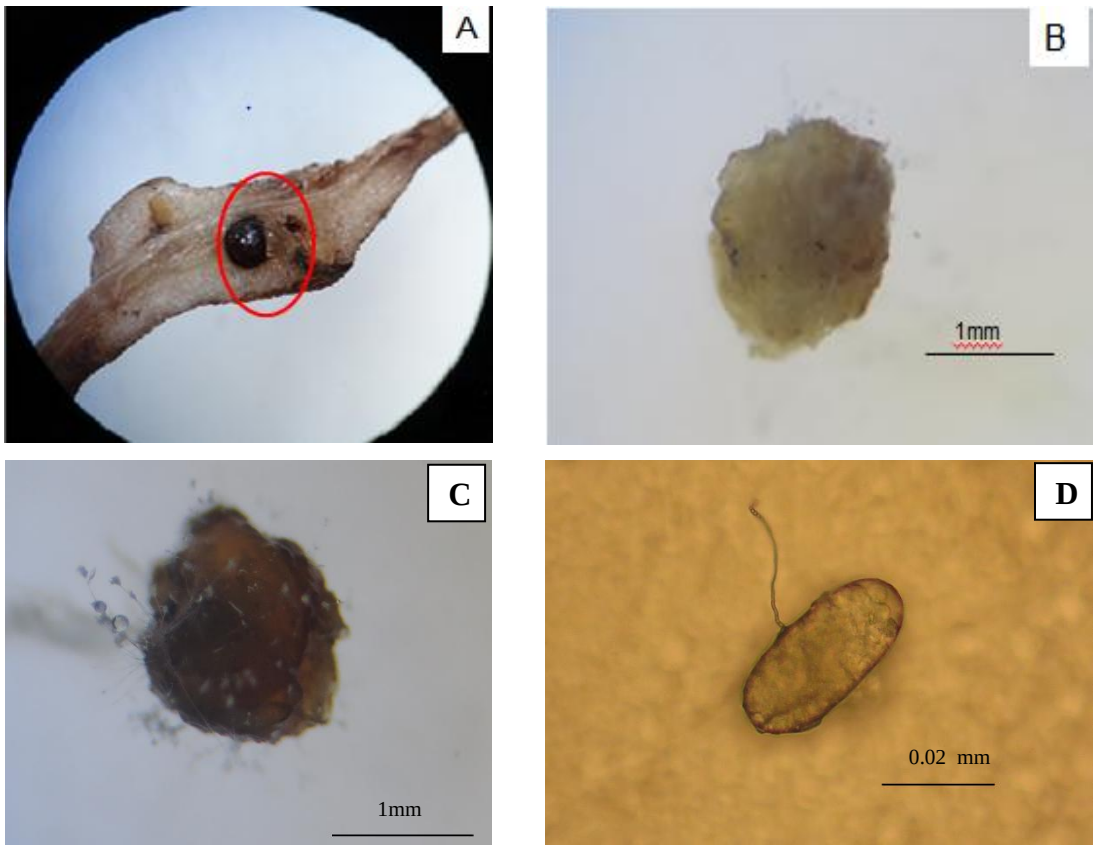

Figure 4. (A) Egg mass of *Meloidogyne* sp., (B) Before the egg mass was parasitized, (C) after the egg mass was infected with *P. lilacinus* the large number of condiophores radiating from the egg mass surface, the bar represents 1 mm. (D) Egg was infected by *P. lilacinus* with fungal hyphae radiating from the egg.

The time of 8 strains will be infected on egg masses longer than female nematode (Table 5). Egg mass comprised of many eggs which were captured by gelatinous masses with a glycoprotein matrix (produced by rectal glands in the female, keeping the eggs together and protecting them against environmental extremes and predation). In addition to provide some protection to the eggs from environmental extremes, it is demonstrated that the matrix has antimicrobial properties.

The rates of parasitizing egg masses nematodes after 5 days of incubation reached 50 % of QT5 strain while the rates of parasitizing nematodes after 2 days of incubation exhibited 50 %. Three strains of PB 1.3, PB 2.10 and QT5 exhibited 83.33 %, 75 % and 75 % parasitism on egg masses after 11 days of incubation. The reported data in table 5 show that, three strains of PB 1.3, PB 2.10 and QT5 had effect on parasitic egg masses very well (Figure 4).

4. CONCLUSION

The ability to degrade chitin of PB 3.3, PB 2.9, QT2, and QT5 were high while PB 2.10, PB 1.3, KL5, and KL6 strains could efficiently secrete protease.

Four strains of PB 2.10, PB 1.3, KL6 and QT5 belonged to the first group achieved the highest parasitic (> 90 %) effects on female after 3 days of incubation. Three strains of PB 1.3, PB 2.10 and QT5 had effect on parasitic egg masses very well.

Strains from forest soils had the ability to parasitizing eggs masses and female nematodes higher than strains from black pepper rhizospheres *in vitro.*

Acknowledgement: This study was funded by annual budget of Biotechnology Center of Ho Chi Minh City. We would like to show gratefullness to Pham Nguyen Duc Hoang, Vo Thi Thu Oanh, Le Thi Mai Cham and Le Thuy Nhi who have provided insights and leadership to assist in this work.

REFERENCES

1. Nguyen N. C. and Nguyen V. T. - Faunna of Vietnam – Volume 4: Plant parasitic nematodes in Vietnam, Sience and Technics Publishing House (2000) 1–401, (in Vietnamese).
2. Pendse M. A., Karwande P. P. and Limaye M. N.- Past, present and future of nematophagous fungi as bioagent to control plant parasitic nematodes, The Journal of Plant Protection Sciences **5** (2013) 1–9.
3. Jatala P., Tenbach K. R. and Bocangel M.- Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes, Journal of Nematology **11** (1979) 303.
4. Luangsa-ard J., Houbraken J., Doorn V. T., Hong S. B. and Borman M. A., Hywel-Jones L.N. and Samson A. R.- *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*, Federation of European Microbiological Societies Microbiol Lett **321** (2013) 141–149.
5. Khan A., Williams L. K. and Nevalainen K. M. H. - Infection of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum*, BioControl **51** (2006) 659–678.
6. Moosavi M. R. and Zare R. - Chapter 4 Fungi as Biological Control Agents of Plant-Parasitic Nematodes, In Plant Defence: Biological Control (2012) 67–107.
7. Fitters P. F. L. and Den Belder E.- A time-lapse technique to study the effect of fungal products on embryogenesis of nematode eggs, Meded Fac Landbouwwet Univ Gent **58** (2b) (1993) 751–756.
8. Bergmeyer H. U, Methods of enzymatic analysis 2, Wiley-Blackwell (1974) 1018–019.
9. Shimahara K. and Takiguchi Y.- Preparation of crustacean chitin, Methods Enzymol **161** (1988) 417–423.
10. Hussey R. S., Barker K. R. - A comparison of methods of collecting inocula for *Meloidogynes* pp., including a new technique, Plant Dis. Rep. **57** (1973) 1025–1028.
11. Suna M. H., Gao L., Shi Y. X., Li B. J., Liu X. Z. - Fungi and actinomycetes associated with *Meloidogyne* spp. eggs and females in China and their biocontrol potential, Journal of Invertebrate Pathology **93** (2006) 22–28.
12. Nguyen V. N., Kim Y. J., Oh K. T., Jung W., Park R. D. - The role of chitinase from *Lecanicillium antillanum* B-3 in parasitism to root-knot nematode *Meloidogyne incognita* eggs, Biocontrol Science and Technology **17** (2007) 1047–1058.