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EFFICACY OF ENTOMOPATHOGENIC FUNGI AGAINST *Spodoptera litura* (Lepidoptera: Noctuidae) AND APHIDS *Myzus precise* (Hemiptera: Aphididae) ON VEGETABLES

Trinh Thi Xuan[1*], Tran Van Hai[1], Pham Kim Son[1]
[1]Department of Plant Protection, College of Agriculture & Applied Biology, Cantho University, Vietnam
*Corresponding Author E-mail: trinhthixuan@ctu.edu.vn

Abstract: Some entomopathogenic agents isolated from fungi-infected insects on vegetables were identified: *Metarhizium anisopliae* Sorokin (Ma), *Beauveria bassiana* Vuillemin (Bb), Verticillium sp. (Ver), *Paecilomyces* sp. (Pae), and *Nomuraea rileyi* Samson (Nr) at the Plant Protection Department (PPD), Can Tho University (CTU). Between the media of PDA, CDA, SDAY1, and SDAY2, the medium of SDAY2 is the most favorable for developing the diameter of the conidial colonies of Ma, Bb, Ver, Pae, and gives the maximum amount of conidia at 14 days after inoculation. Between the media of PMA, PMAY, SMAY1, SMAY2; the medium of PMAY leads to the maximum amount of conidia at 21 days after inoculation. For the spongy fermentation method, the Method3 (and Method4 may be used in production of *Metarhizium anisopliae*. Under the laboratory condition; Ma, Bb and Nr may effectively control the armyworm (*Spodoptera litura* Fab.) at 10^8conidia.ml^{-1} suspension. Under the laboratory condition; at 10^8conidia.ml^{-1} suspension, Ma and Bb also give high efficacy to control the aphids (*Myzus percicae* Sulzer). The corrected mortalities were 71.9% and 79.1%, respectively.

Key words: *Entomopathogenic agents, Insect, Vegetable*

INTRODUCTION

In the Mekong Delta of Vietnam, the primary measure to control insects on vegetables usually depends on applying chemical pesticides. However, insecticidal control has led to several problems in agricultural production like insecticide resistance, pest resurgence, undesirable toxic effects to natural enemies of pests, and toxic residues in crops. Therefore, the search for biocontrol methods of entomopathogenic fungi has shown considerable promise. This research aims looking for some entomopathogenic agents that may control armyworms (*Spodoptera litura* Fab.) and aphids (*Myzus persicae* Sulzer) on vegetables.

MATERIALS & METHODS

1. Isolation of entomopathogenic fungi:

Fungi-infected cadavers of armyworm, aphid were collected from vegetable fields and isolated on Potato-Dextrose-Agar (PDA) medium. All the entomopathogenic agents were identified by the keys of Barnett and Barry (1972) and other papers.

2. For the screening of favorable medium of entomopathogenic fungi; *Metarhizium anisopliae, Beauveria bassiana, Verticilium* sp. and *Paecilomyces* sp. were cultured on four different medium such as PDA, Czapek-Dox-Agar (CDA), Sabouraud-Dextrose-Agar- Yeast (SDAY1) and Sabouraud-Dextrose-Agar-Yeast mixed with some microelements (SDAY2) under the room condition of temperature (25-28^{0}C), humidity (65-78%). The colony diameter of fungi was measured after a certain time after inoculation.

3. Identification of the duration of development of entomopathogenic fungi:
The about fungi were also cultured on four different medium: PDA, CDA, SDAY1 and SDAY2. The amount of conidia in each medium was measured after inoculation of 7, 14, 21 and 28 days.

4. Identification of the most favorable medium for development of *Nomuraea rileyi*:
This fungus was cultured on four different media such as: Potato-Maltose-Agar (PMA), Potato-Maltose-Agar-Yeast (PMAY), Sabouraud-Maltose-Agar-Yeast (SMAY1) and Sabouraud-Maltose-Agar-Yeast mixed with some microelements (SMAY2). The amount of conidia in each medium was also measured after inoculation of 21, 28 and 35 days.

5. Testing the products of spongy fermentation methods:
The *Metarhizium anisopliae* was multiplied on four different formulations of medium in the flask under the room condition of laboratory:

Spongy fermentation methods	Formulation (g or ml)					
	Rice husk	Rice bran	Maize	Soybean	Distill water	Pepton
Method1	50	20	15	15	50	0
Method2	50	20	15	15	50	1
Method3	50	10	20	20	50	0
Method4	50	10	20	20	50	1

After 10 days of fermentation, the fermented products were collected and dried in the oven at 35-40^{0}C. Later, they were pulverized into powder. The alive conidia of product were tested by counting the colony forming unit (CFU) on the surface of medium in the Petri dish under laboratory condition.

6. Efficacy of some entomopathogenic fungi on army worms and aphids
-Under the room conditions of the laboratory, each treatment was conducted with 30 larvae of the second instars of armyworms. The experiment was laid out in a RCD with 4 replications. All the larvae were dipped into the conidial suspension of entomopathogenic

fungi (10^8 conidia.ml^{-1} + 0.05 % Tween 20) in 30 seconds. The mortality of army worm was measured at the time of 3, 5, 8 and 11 days after treatment (DAT).

-Each treatment was conducted with 60 larva of 1-2 instars aphids. The experiment was laid out in a RCD with 4 replications. All the aphids living on vegetable leaves in the Petri dish were sprayed directly by conidial suspension of entomopathogenic fungi (10^8 conidia.ml^{-1} + 0.05 % Tween 20) in 30 seconds. The mortality of aphids was measured at the time of 11 DAT.

7. For testing the efficacy of *Nomuraea rileyi* on army worms under the condition of laboratory, the treatment was conducted with 30 larva of second instars at two different concentration: (10^8 conidia.ml^{-1} + 0.05 % Tween 20) and (10^7 conidia.ml^{-1} + 0.05 % Tween 20). The experiment was laid out in a RCD with 4 replications. All the larvae were dipped into the conidial suspension of entomopathogenic fungi in 30 seconds. The mortality of army worm was measured at the time of 3, 5, 8 and 11 days after treatment.

RESULTS AND DISCUSSIONS

3.1. Based on the keys of Barnett and Barry (1972), the entomopathogenic agents which isolated from naturally infected insects were *Metarhizium anisopliae* Sorokin, *Beauveria bassiana* Vuillemin, *Verticilium* sp., *Paecilomyces* sp. *and Nomuraea rileyi* Samson.

3.2. To screen the favorable medium for the development of entomopathogenic fungi, Table 1 indicated that the colony diameter of Ma which was the longest on SDAY2 (7.3 cm) significantly differed from these others in the same row: CDA (6.9 cm), SDAY1 (6.1 cm) and PDA (3.8 cm). The colony diameters of Bb on CDA (5.0 cm), PDA (4.7 cm), SDAY2 (4.6cm) were similar and significantly differed from SDAY1 (3.8 cm) in the same row. The colony diameters of Ver on SDAY1 (6.0 cm) and SDAY2 (6.0 cm) were significantly different from CDA (5.3 cm) and PDA (5.3 cm) in the same row. The colony diameters of Pae on SDAY2 (6.8 cm) also significantly differed from SDAY1 (6.5 cm), CDA (6.2 cm) and PDA (5.3 cm) in the same row. These results were in accordance with the studies of Pham Thi Thuy (1994).

Table 1. Effect of different media on the development of colonies of entomopathogenic fungi

T = 28^{o}C, H = 65%

Treatment	Colony diameter (cm)				
	PDA	CDA	SDAY1	SDAY2	CV (%)
Ma	3.8 c	6.9 b	6.1 b	7.3 a	7.2
Bb	4.7 b	5.0 b	3.8 a	4.6 b	5.8
Ver	5.3 a	5.3 a	6.0 b	6.0 b	5.1
Pae	5.3 a	6.2 b	6.5 b	6.8 c	2.8

Means followed by a common letter in the same row are not significantly different at 5% level by DMRT.

3.3. For identification of duration on development of entomopathogenic fungi, Table 2 showed that at the time of 14 days after inoculation, the conidial number of Ma on SDAY2 (24.7 x 10^8 conidia.ml^{-1}), SDAY1 (24.6 x 10^8 conidia.ml^{-1}), CDA (21.7 x 10^8 conidia.ml^{-1}) were not significantly different, but significantly differed from PDA (7.2 x 10^8 conidia.ml^{-1}) in the same row. The conidial number of Bb on SDAY2 (12.1 x 10^8 conidia.ml^{-1}) was significantly different from SDAY1 (6.2 x 10^8 conidia.ml^{-1}), PDA (5.0 x 10^8 conidia.ml^{-1}) and CDA (4.5 x 10^8 conidia.ml^{-1}); these treatments might be significantly differentiated in the same row. The conidial number of Ver on SDAY2 (2.2 x 10^8 conidia.ml^{-1}) was significantly different from SDAY1 (1.9 x 10^8 conidia.ml^{-1}), CDA (0.5 x 10^8 conidia.ml^{-1}) and PDA (0.4 x 10^8 conidia.ml^{-1}). The conidial number of Pae on SDAY2 (1.9 x 10^8 conidia.ml^{-1}), SDAY1 (1.4 x 10^8 conidia.ml^{-1}), CDA (0.6 x 10^8 conidia.ml^{-1}) and PDA (0.2 x 10^8 conidia.ml^{-1}) were also significantly different in the same row.

Table 2. Effect of different media on the establishment of conidia of entomopathogenic fungi at 14 days after inoculation

Room: T = 28^{o}C, H = 65%

Treatment	Conidial number (x10^8 conidia.ml^{-1})				
	PDA	CDA	SDAY1	SDAY2	CV (%)
Ma	7.2 b	21.7a	24.6a	24.7a	0.8
Bb	5.0 b	4.5 a	6.2 c	12.1d	1.6
Ver	0.4 a	0.5 b	1.9 c	2.2 d	0.2
Pae	0.2 a	0.6 b	1.4 c	1.9 d	0.3

Means followed by a common letter in the same row are not significantly different at 5% level by DMRT.

In Table 3, at 28 days after inoculation, all the conidial numbers of entomopathogenic fungi were always lower than those in Table 2. The conidial number of Ma on SDAY2 (16.7 x 10^8 conidia.ml^{-1}), SDAY1 (13.7 x 10^8 conidia.ml^{-1}), CDA (12.6 x 10^8 conidia.ml^{-1}) and PDA (6.3 x 10^8 conidia.ml^{-1}) were not significantly different in the same row. Otherwise, the conidial number of Bb on SDAY2 (5.8 x 10^8 conidia.ml^{-1}), of Ver on SDAY2 (0.8 x 10^8 conidia.ml^{-1}) and Pae (0.1 x 10^8 conidia.ml^{-1}) always highest and significantly different in comparison with other medium in the same row. It may be mentioned that Sabouraud-Dextrose-Agar-Yeast mixed with some microelements (SDAY2) is the favorable medium for development of these entomopathogenic fungi.

Table 3. Effect of different media on the establishment of conidia of entomopathogenic fungi at 28 days after inoculation

Room: T = 28^{o}C, H = 65%

Treatment	Conidial number (x10^8 conidia.ml^{-1})				
	PDA	CDA	SDAY1	SDAY2	CV (%)
Ma	6.3 a	12.6 a	13.7 a	16.7 a	2.3
Bb	1.7 a	1.9b	3.9 c	5.8 d	0.7

Ver	0.3 a	0.4 b	0.6 c	0.8 d	0.1
Pae	0.1 a	0.4 b	0.1 c	0.1 d	0.3

Means followed by a common letter in the same row are not significantly different at 5% level by DMRT.

3.4. For identification of the most favorable medium for development of *Nomuraea rileyi,* Table 4 showed that conidial number of *N. rileyi* was highest at the time of 21 days after inoculation; these data gradually reduced until 35 days. At the time of 21 days after inoculation, the PMAY gave the highest conidial number (48 x 10^8 conidia.ml^{-1}), and was significantly different in comparison with other medium: PMA (12 x 10^8 conidia.ml^{-1}), SMAY1 (35 x 10^8 conidia.ml^{-1}) and SMAY2 (35 x 10^8 conidia)) in the same column. This result was similar to the research of Gardner (1994), who reported that PMAY is the most favorable medium for the development of *Nomuraea rileyi.*

Table 4. Effect of different media on the establishment of conidia of *Nomuraea rileyi*

Room: T = 25^{o}C, H = 80%

Treatment	Conidial number (x10^8 conidia.ml^{-1})		
	21 DAT	28 DAT	35 DAT
PMA	12 a	9 a	7 a
SMAY1	35 b	27 b	14 b
SMAY2	35 b	33 b	17 b
PMAY	48 c	46 c	23 c
CV (%)	0.4	0.6	0.8

Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

3.5. For the production of *Metarhizium anisopliae* as a product by spongy fermentation methods, Table 5 showed that after 10 days of inoculation, the total number of conidia of Method3 (30.5 x 10^8 conidia.g^{-1}) and Method4 (35.8 x 10^8 conidia.g^{-1}) were very high. The CFU of Method3 (16.5 x 10^8 conidia.g^{-1}) and Method4 (18.8 x 10^8 conidia.g^{-1}) were also relatively high. The recovery of conidia ratio (CFU/ Total number of conidia) on Method3 and Method4 was 54.1% and 52.5%, respectively. Generally, the Method3 and Method4 may be used in the production of *Metarhizium anisopliae.*

Table 5. Recovery of *Metarhizium anisopliae* as product by spongy fermentation methods

Room: T = 27^{o}C, H = 69%

Spongy fermentation method	Total number of conidia (x 10^8 conidia.g^{-1})	CFU (x10^8 conidia.g^{-1})	CFU/ Total number of conidia (%)

Method1	28,1 ab	7,8 a	27,6
Method2	25,8 a	10,9 a	42.1
Method3	30,5 ab	16,5 ab	54,1
Method4	35,8 b	18,81 b	52,5
CV (%)	6.5	4.9	

Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

3.6. For testing the efficacy of some entomopathogenic fungi on army worms under the condition of laboratory, Table 6 indicated that at the time of 11 DAT of 10^8conidia.ml^{-1} suspension, the mortality of army worms killed by Ma (64.1%) and Bb (68.7%) were similar and significantly different from Pae (39.9%), Ver (20.8%) and non-treated control (6.6%). This table also showed that at the time of 11 DAT of the same concentration of conidial suspension, the mortality of aphids killed by Ma (82.9%) and Bb (90.1%) were similar and significantly different from Pae (64.9%), Ver (30.6%) and non-treated control (11.0%).

Table 6. Mortality of army worms and aphids killed by entomopathogenic fungi

T = 27^{o}C, H = 71%

Treatment	Mortality (%) at 11 days after inoculation	
	Army worm	Aphid
Ma	64.1 c	82.9 c
Bb	68.7 c	90.1 c
Pae	39.9 b	64.9 b
Ver	20.8 a	30.6 a
Non-treated control	6.6 a	11.0 a
CV (%)	14.4	19.2

Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

For testing the efficacy of *Nomuraea rileyi* on armyworms under laboratory conditions, Table 7 shows that larvae mortality gradually increased from 3 to 11 DAT. At 11 days, the concentration of Nr (108 conidia.ml-1) gave the highest mortality (63.6%), which was significantly different from the concentration of Nr (10^7 conidia.ml^{-1}) and non-treated control (40.6% and 6.6%, respectively).

Table 7. Mortality of armyworms killed by *Nomuraea rileyi* at two different conidial suspension

T = 27^{o}C, H = 71%

Treatment	Mortality (%) after inoculation at			
	3 days	5 days	8 days	11 days
Nr (10^8 conidia/ml)	8.6	35.3 b	43.3 b	63.6 c
Nr (10^7 conidia/ml)	6.6	30.6 b	33.3 b	40.6 b
Non-treated control	5.3	5.9 a	6.6 a	6.6 a
CV (%)	12.6	22.3	20.6	17.9
	ns	*	*	*

Means followed by a common letter in the same column are not significantly different at 1% level by DMRT. ns: non significant; * : significant

CONCLUSION

-Five entomopathogenic agents isolated from fungi-infected insects on vegetables were identified: *Metarhizium anisopliae* Sorokin, *Beauveria bassiana* Vuillemin, *Verticillium* sp. , *Paecilomyces* sp. and *Nomuraea rileyi* Samson.
-Between the media of PDA, CDA, SDAY1 and SDAY2; Medium of SDAY2 is the most favorable to develop diameter of the conidial colonies of Ma, Bb, Ver, Pae.
-Between the media of PMA, PMAY, SMAY1 and SMAY2; the medium of PMAY leads to the maximum amount of conidia.
-Method3 and Method4 may be used in production of Ma by the spongy fermentation method.
-Ma, Bb and Nr may effectively control the armyworm *(Spodoptera litura* Fab.*)* at 10^8conidia.ml^{-1} suspension.
-Ma and Bb also give high efficacy to control the aphids *(Myzus percicae* Sulzer*)* at 10^8conidia.ml^{-1} suspension. The corrected mortalities were 71.9% and 79.1%, respectively.

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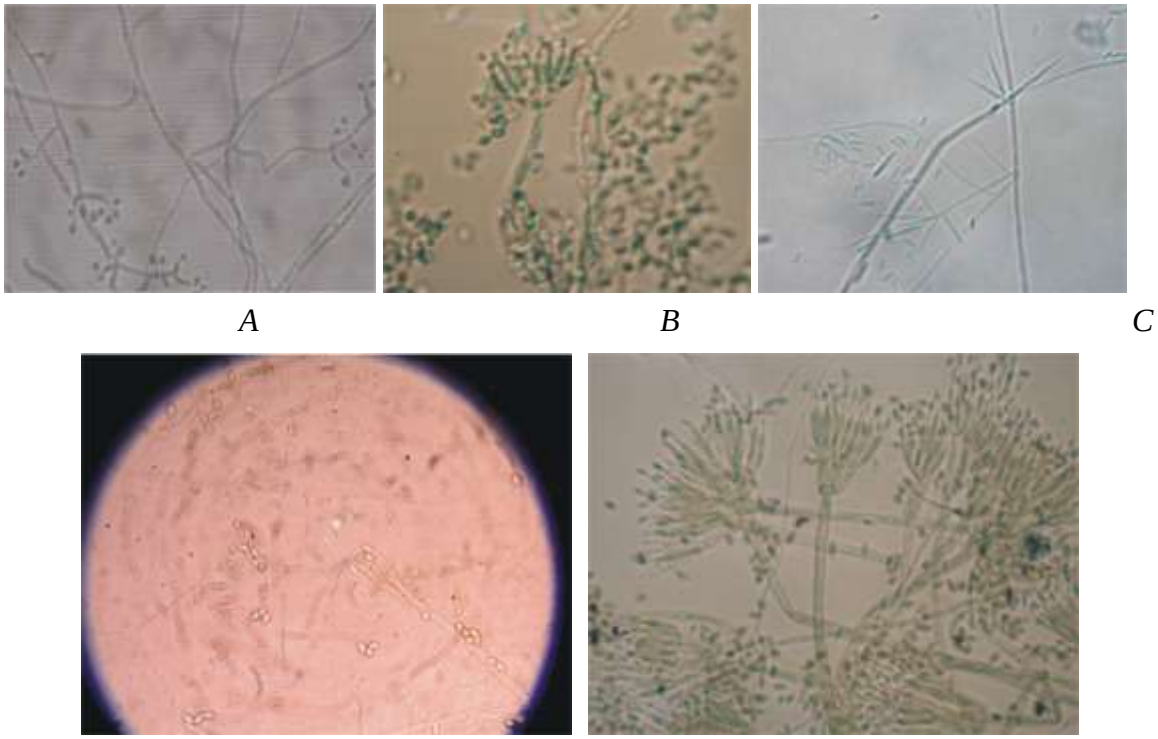
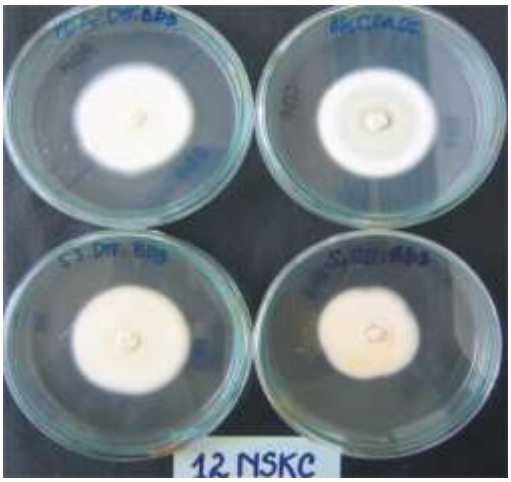


A *B* *C*

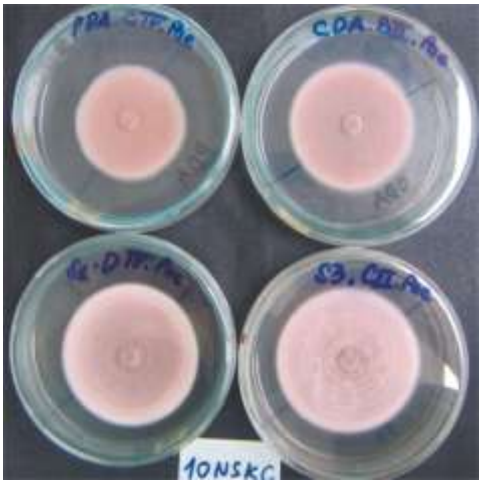
D *E*

Picture 1: Entomopathogenic fungi observed under microscope

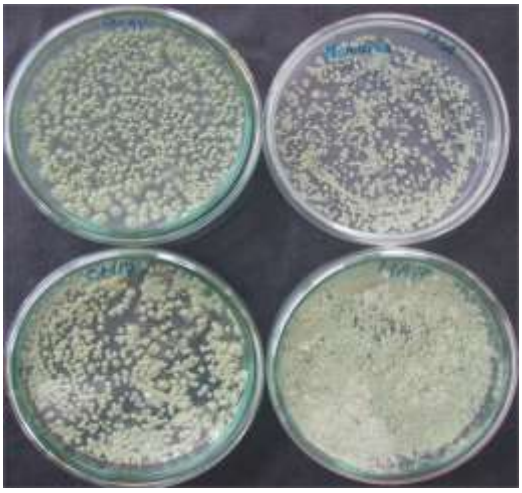
A: Beauveria bassiana; B: Nomuraea rileyi; C: Verticillium sp.; *D: Metarhizium anisopliae; E: Paecilomyces* sp.



Picture 2: Development of Bb on different media



Picture 3: Development of Pae on different media



Picture 4: Development of Nr on different media

Picture 5: Development of Ver on different media

Picture 6: Insect cadavers killed by entomopathogenic fungi

a: Army worm infected by Nr b: Army worm infected by Ma c: Aphids infected by Ma