Phangthavong. (2023). Souphanouvong University Journal of Multidisciplinary Research and Development, ISSN 2521-0653. Volume 9. Issue 2. Apr – Jun 2023. Page 291 – 302

The Time-Concentration-Mortality Modeling and Virulence Indices for Strain NGS71814 of *Metarhizium anisopliae* against the Potato Tuber Moth (PTM), *Phthorimaea operculella*

Souksamone Phangthavong[1], Bin Chen[*, 2], Yaqiang Zheng[2], Siyu Liu[2], Chanhom Loiheuang[3], Kita Chanthanouvong[1], Vongpasith Chanthakhoun[1], Bounxian Phetlumphun[1], Anousith Vannaphon[1]

Department of Forest resource, Faculty of agriculture and forest resource, Souphanouvong University, Lao PDR

***[2]Correspondence:** Chen Bin, State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, College of Plant Protection, Yunnan Agricultural University, Kunming, People's Republic of China. Tel: +86 13708876067, Email: chbins@163.com*

[1]Department of Forest resource, Faculty of agriculture and forest resource, Souphanouvong University, Lao PDR, Tel: +85620 54549636, Email: souksamonep@hotmail.com

[3]Department of Biology, Faculty of Natural Science, National University of Laos, Lao PDR

Article Info:

Submitted: Mar 14, 2023

Revised: May 11, 2023

Accepted: Jun 17, 2023

Abstract

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) attack solanaceous crops worldwide. They cause high economic losses. *Metarhizium anisopliae* is an important entomopathogenic fungi. To determine the effectiveness of this fungus as a biocontrol agent for PTM, virulence of *M. anisopliae* on 1st, 2nd and 3rd instar larvae, and pupae were conducted, the resulting data were analyzed using a time-dose-mortality modeling technique, yielding the parameters for time and dose effects of the *M. anisopliae*. The fungi *M. anisopliae* caused 95%, 100%, 100% and 100% mortality of 1st, 2nd and 3rd instar larvae, and pupae at conidial concentration of 2×10^8 conidia/mL. The estimated parameter (β) for the concentration effect of *M. anisopliae* was 0.596, 0.66, 0.282 and 1.137 in bioassay 1st, 2nd, 3rd instar larvae, and pupae. Which indicated that the pathogenicity of *M. anisopliae* against the pupae were greater than that of larvae. Moreover, the *t* statistics for all parameters estimated were significant ($P < 0.01$). At 2×10^8 conidia/mL concentration, 3rd and 2nd instar larvae had short lethal times (LT_{50}) of 1.01 and 1.3 d compared with 3.5 and 3.6 d for pupae. The values of LC_{50} estimated on days 1 to 4 after exposure decreased from 2.27×10^8 to 4.64×10^1 conidia/mL for the 3rd instar larvae, on days 3 - 10 decreased from 1.51×10^5 to 8.45×10^2 conidia/mL for the 2nd instar larvae, on days 3 - 10 decreased from 1.99×10^6 to 2.43×10^4 conidia/mL for the 1st instar larvae, and on days 1 - 7 decreased for pupae. The estimated of LC_{90} during the same time period decreasing from 4.07×10^{12} to 8.32×10^5 conidia/mL. Finally, the LC_{90} decreasing to 3.21×10^4 conidia/mL until the day 10 for the 3rd instar larvae, decreasing on days 4 - 10 from 2.52×10^6 to 5.25×10^4 conidia/mL for the 1st and 2nd instar larvae and decreasing to 2.47×10^6 conidia/mL on the day 7 for pupae.

Keywords: *Potato tuber moth, Phthorimaea operculella, Metarhizium anisopliae, Virulence bioassays.*

1. Introduction

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) attack solanaceous crops worldwide (Liu et al., 2018) with potato being favored (Sabbour, 2015). In addition, it is considered the most damaging insect pest of potatoes in developing countries in the tropics and subtropics regions. Including potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.) (Rondon, 2010), chili (*Capsicum annum),* wild brinjal (*Solanum nigrum)* and Datura stramonium (Trivedi & Rajagopal, 1992). This insect is a serious insect pest of potato (*Solanum tuberosum* L.) both during storage and in the field (Sporleder et al., 2004). In addition, they cause high economic losses, because of its closed relationship with the host plant and high reproductive potential (Rondon, 2010). PTM adult female deposit eggs in potato foliage or near to tubers, while the larvae mine leaves, stems, and petioles and excavate tunnels through potato tubers (Rondon et al., 2007). They sometimes oviposit in the soil if potato foliage is unavailable (Anfora et al., 2013; Rondon, 2010). After the potato is harvested, the insect may continue to develop on tubers or volunteer plants remaining in the field including other solanaceous plants such as tomatoes (Gilboa & Podoler, 1995). *P. operculella* was first detected in the United States in California in 1856, and since then it has been a pest of potatoes in southern California and other southern states (Graf, 1917).

Entomopathogenic fungi infect insects through the cuticle, grow as hyphal bodies or hyphae in the hemocoel, and cause host death by nutritional destruction of tissues and producing toxic metabolites and pathogenic enzymes (Kikuchi et al., 2009). *Metarhizium flavoviride*, have been used as biological insecticides (Sabbour & Singer, 2015). Adult desert locusts inoculated with *M. flauouiride* died after a period ranging from 5 days (Moore et al., 1992). To review the insect fungi on PTM, and to provide that information on the virulence of them against the larvae or pupae of PTM, so as to show that present study is very necessary and important. Therefore, the objective of the present study was to test the virulence of the strain NGS71814 of *M. anisopliae* isolates from the cadavers of Lepidoptera larvae by bioassays with PTM 1st, 2nd, 3rd instar larvae, and pupae, and also to get a better understanding of pathogenicity of *M.anisopliae* to larvae and pupae of PTM. The best bioassay approach could potentially be developed for use in integrated pest management (IPM) strategies against PTM in the future.

2. Materials and methods

2.1 Insect colony

Standard laboratory colony of the PTM was reared on potato tubers *Solanum tuberosum* as a natural host plant in the cages (W × L × H = 30 cm × 30 cm × 40 cm) under controlled conditions (25 ± 2 °C and 85 ± 5% R.H). Moths were kept in oviposition cages that consist of glass (3 cm in diameter and 8 cm in height), covered with nylon mesh (D = 3 cm, diameter) and fed by 5% honey solution (v/v) for eggs laying. The eggs laid on the nylon mesh were collected daily and transferred to Petri-dishes until larval hatch. The larvae were carefully transferred to the cages containing potato tuber for reared for developed to pupae and adult stages. The pupae were individually kept in Petri-dishes (D = 9 cm) lined with sterilized moisture cotton until adult emergence.

2.2 Fungal isolation and conidial suspension preparation

The fungal isolates used in the study were strain NGS71814 of *M. anisopliae* isolates collected from cadavers of Lepidoptera larvae found in the Xishuangbanna National Nature Reserve in Yunnan Province, China in 2017 (Zheng al et., 2019) and kept in refrigerator at 4 °C in the insect fungi laboratory, college of Plant Protection, Yunnan Agricultural University. *M. anisopliae* NGS71814 was cultured on potato dextrose agar (PDA) medium at 25 ± 2℃ and RH 85 ± 5% relative humidity, and 16 h light: 8 h dark, resulting in the production of a large number of conidia after 7 days old culture. and diluted to prepared concentrations of 2×10^8, 2×10^7, 2×10^6, 2×10^5, 2×10^4 and 2×10^3 conidia/mL in 0.05% (w/v) Tween 80. Conidial were quantified via direct counting using an optical microscope with a Neubauer chamber. Conidial suspensions were serially diluted for subsequent experiments.

2.3 Virulence screening bioassays

The bioassay was carried out in laboratory at Collage of Plant Protection, Yunnan Agricultural University. China. Bioassay was conducted by using immersion (virulence of fungus against the PTM larvae and pupae) with six conidial concentrations of 2×10^8, 2×10^7, 2×10^6, 2×10^5, 2×10^4 and 2×10^3 conidia/mL and a corresponding control (sterile distilled water containing 0.05% TWEEN® 80) were prepared (mortality, 10 days for larvae mortality and 7 days for pupae mortality).

Early 20 individuals healthy of 1st, 2nd and 3rd instar larvae were selected to introduced into each suspension (10 ml) for 10 second, then put them out and put them on a sterilized filter paper for air-drying. Then, the inoculated larvae were put on sterilized fresh leaves of potatoes. Then, the inoculated larvae were put into the Petri dishes (D = 9 cm) lined by filter paper for absorbed moisture, and then were placed in incubators setup temperature at of 25 ± 2℃ and RH 85 ± 5% relative humidity, and 16 h light: 8 h dark. All experiments were repeated 3 times. Larvae mortality was assessed daily until 10 days after exposure.

20 healthy PTM pupae one day old without silk cocoons were randomly assigned to each conidial concentration, with each treatment replicated 3 times. The selected pupae were dipped in the relevant conidial suspension for 10 second, dried on a filter paper separately, and transferred to tissue culture plates lined with moist sterilized cotton and lined with filter paper again for absorbed moisture. and then were placed in incubators setup at temperature of 25 ± 2 ℃ and RH 85 ± 5% relative humidity, and 16 h light: 8 h dark. All experiments were repeated 3 times. Pupae mortality was assessed daily until 7 days after exposure.

2.4 Statistical analysis

For the single-concentration assays, total PTM larvae and pupae mortality on the 10th days and 7th days after treatment was compared. Mortality data were first subjected to angular transformation and then subjected to analysis of variance (ANOVA) ($\alpha = 0.05$). Dunnett's one-tailed *t*-test ($\alpha = 0.05$) was used to detect differences in mortality between isolates and the controls. Isolates that were significantly different from the controls were subjected to a second ANOVA and Dunnett's *t*-test against the standard isolate ($\alpha = 0.05$) (Liu et al., 2002).

The resulting Time - concentration mortality (TCM) data of *M.anisopliae* NGS71814 against PTM larvae and pupae were analyzed by the complementary log-log TCM modeling technique (Robertson & Preisler, 1992; Xu & Feng, 2000). PTM larvae and pupae mortality (q_{ij}), referred to as conditional mortality probability, caused by conidial suspension of a given concentration (d_i) at a specific time interval [t_{j-1}, t_j] (i.e., days 1-10 for

larvae and days 1-7 for pupae after inoculation) was corrected using background mortality (mortality in only sterilized 0.05% TWEEN® 80) observed in the controls of each suspension concentration. It was then fitted to the condition TCM modeling q_{ij}= 1 exp[-exp(γ_j + βlog10(d_i))] (1), by a maximum likelihood equation $\prod_{j=1}^{J}\prod_{i}^{I} q_{ij}^{rij}(1-q_{ij})^{nij-rij}$ (2).

Where n_{ij} represents the number of PTM larvae and pupae surviving at time t_{j-1} after a inoculation dose d_i and r_{ij} represents the number of PTM pupae that died of *M. anisopliae* infection by time interval [t_{j-1},t_j]. β and γ_j were used to determine the cumulative TCM relationships in the form of p_{ij} = 1-exp[-exp(τ_j + βlog10(d_i))] (3) were then obtained with $\tau_j = \ln(\sum_{k=1}^{j} e^{\gamma\kappa})$. The result of β and τ_j were estimated and their variance and covariance were used to calculate lethal concentrations (LC), including LC_{50} and LC_{90} (the concentration of *M. anisopliae* causing 50 or 90% of the PTM larvae and pupae to die) (Zheng al et., 2019). The analysis processes were conducted by the software of Data Processing System (Tang & Feng, 2010).

3. Results

3.1 Infection of PTM larvae and pupae by fungal *M. anisopliae*

3.1.1 PTM larvae

The 1st, 2nd and 3rd instar PTM larvae were selected to experiment with six difference suspension concentration of 2 × 10^3 to 2 × 10^8 conidia/mL of *M. anisopliae* NGS71814. The results showed that the inoculation PTM larvae after 12 h, there symptoms of weakness, slowed movement down, food poisoning, and started to die in 24 h after the inoculation or on the days 1 - 3 respectively. Died PTM larvae came to pale yellow cuticle on the 1st day after infection by fungal. The white mycelium of fungal were germination on larvae cuticle on the 2nd day after died (Figure. 1), larvae cuticle were changed to dark on the day 3rd and white mycelium of fungal were distribution on around body on day 4th and 5th, the white mycelium almost germination covered with appeared green spore of fungal on day 6th. Finally, the fungal were completely covered on PTM larvae with a large number of *M. anisopliae* mycelium on 7th day after the infection.

3.1.2 PTM pupae

Fungal were starting germination white mycelium on PTM pupae body cuticle on 2nd day after infection, the cuticle still kept normal dark yellow (Figure. 2). The mycelium of fungal were contributed grow up on pupae cuticle and their cuticle turn dark on the 4th day. The white mycelium were continuous growth up from short to long synnemata and appeared green spore of fungal on some point of pupae cuticle on the 6th day. The white mycelium almost covered on pupae body and appeared many green spores of fungal on pupae joint on the 8th day. Pupae were completely covered by white mycelium and green spore of fungal were growth more continuously on the 10th day. The synnemata gradually elongated with the growth of the fungal and were completely developed by the 14th day after infection. And a large number of conidias were produced on top of the synnemata.

3.2 PTM larvae and pupae mortality

3.2.1 PTM larvae mortality

The 1st, 2nd and 3rst instar PTM larvae were treated with the *M.anisopliae* NGS71814 at concentration of 2 × 10^3 to 2 × 10^8 conidia/mL. On the 1st instar PTM larvae mortality was (95 ± 0.74) % (2 × 10^8 conidia/mL) on day 4th after inoculation and (90 ± 0.36) % (2 ×10^7 conidia/mL) on day 9th. Followed by (86.66 ±

0.04) % (2×10^6 conidia/mL) on day 9th, while 66.66 ± 0.56% (2×10^5 conidia/mL) on day 10th and (58.33 ± 0.60) % (2×10^4 conidia/mL) on day 10th and lowest mortality was (38.33 ± 0.47) % (2×10^3 conidia/mL) on day 10th respectively (Figure. 3A). The final cumulative mortality of PTM larvae on 1st instar on 10 day ranged from 61.66% to 95% in 2×10^8 conidia/mL, 33.33% to 90% in 2×10^7 conidia/mL, 6.66% to 86.66% in 2×10^6 conidia/mL, 3.33% to 66.66% in 2×10^5 conidia/mL, 1.66% to 58.33% in 2×10^4 conidia/mL and 1.66% to 38.33% in 2×10^3 conidia/mL respectively.

On the 2nd instar PTM larvae mortality, showed higher was 100 % (2×10^8 conidia/mL) on day 3rd after inoculation and 100% (2×10^7 conidia/mL) on day 4th, followed by 96.66 ± 0.47% (2×10^6 conidia/mL) on day 4th and 90 ± 0.61% (2×10^5 conidia/mL) on day 8th, while 86.66 ± 0.36% (2.0×10^4 conidia/mL) on day 9th. Among lowest mortality was (73.33±0.60) % (2×10^3 conidia/mL) on day 9th (Figure. 3B). The final cumulative mortality of second instar PTM larvae on 10 days ranged from 50% to 100% in 2×10^8 conidia/mL, 16.66% to 100% in 2×10^7conidia/mL, 6.66% to 96.66% in 2×10^6 conidia/mL, 3.33% to 90% in 2×10^5 conidia/mL, 5% to 86.66% in 2×10^4 conidia/mL and 3.33% to 86.66% in 2×10^3 conidia/mL.

On the 3st instar PTM larvae mortality was 100% (2×10^8 conidia/mL) on day 5th after inoculation, 100 ± 0.19% ($2 \times 1\ 0^7$ conidia/mL) on day 5th, 98.33 ± 0.47% (2×10^6 conidia/mL) on day 5th, 93.33 ± 0.68% (2×10^5 conidia/mL) on day 7th, (90 ± 0.30) % (2×10^4 conidia/mL) on day 7th and (83.33 ± 0.41) % (2×10^3 conidia/mL) on day 7th (Figure. 3C). The final cumulative mortality of 3rd instar PTM larvae on 10 days ranged from 53.33% to 100% in 2×10^8 conidia/mL, 48.33% to 100% in 2×10^7conidia/mL, 33.33% to 98.33% in 2×10^6 conidia/mL, 23.33% to 93.33% in 2×10^5 conidia/mL, 13.33% to 90% in 2.0×10^4 conidia/mL and 8.33% to 83.33% in 2×10^3 conidia/mL.

In general, the results of PTM larvae mortality also showed that in the concentration of 2×10^8 and 2×10^7 conidia/mL on PTM 1st, 2nd and 3rd instar larvae there was no significantly difference observed in mean percentage mortality ($F = 100.5$, $P < 0.05$ and $F = 163.5$, $P < 0.05$). among there were significantly results mortality in the concentration of 2×10^6, 2×10^5, 2×10^4 and 2×10^3 conidia/mL ($F = 28.5$, $P < 0.05$; $F = 14.33$, $P < 0.05$; $F = 25$, $P < 0.05$ and $F = 3$, $P > 0.05$).

3.2.2 PTM pupae mortality

Final averaged mortality of PTM pupae in the treatment on 7 days in six differences concentration from 2×10^3 to 2×10^8 conidia/mL was (15 ± 0.34) %, (28.33 ± 1.16) %, (51.66 ± 1.35) %, (85 ± 0.77) %, 100% and 100% respectively. Dead pupae in the control (Tween 80, 0.05%) did not shown any fungal *M.* mycelium growth on pupae cuticle. Cumulative mortality increased with conidial concentration (Figure. 3D). The mortality was higher in concentration of 2×10^8 conidia/mL and 2×10^7 conidia/mL respectively. The final increasing mortality of PTM pupae on 7 days ranged from 3.33% to 15% in 2×10^3 conidia/mL, 15 to 28.33% in 2×10^4 conidia/mL, 6.67% to 51.66% in 2×10^5 conidia/mL, 16.67% to 85% in 2×10^6 conidia/mL, 3.33% to 100% in 2×10^7 conidia/mL and 5% to 100% in 2×10^8 conidia/mL. From the finally results showed that PTM pupae were highest significant mortality in conidial suspension concentration of 2×10^8 conidia/mL and followed by in conidial suspension concentration of 2×10^7 conidia/mL,

and lowest mortality in conidial suspension concentration of 2×10^3 conidia/mL.

3.3 Fitted TCM relationships on PTM larvae and pupae

The observed responses of PTM mortality fitted the TCM model. Data for all bioassays fit the TCM model with accepted homogeneity fit based on Hosmer - Lemeshow statistic C ($P \geq 0.05$), ($C = 13.46$, $df = 8$, $P = 0.09$ for the 1st instar larvae; $C = 11.91$ $df = 8$, $P = 0.53$ for the 2nd instar larvae; $C = 6.01$, $df = 7$, $P = 0.54$ for the 3rd instar larvae; $C = 1.62$, $df = 8$, $P = 0.99$ for the pupae). Moreover, the *t* statistics for all parameters estimated were significant ($P < 0.0001$). The estimated parameter (β) for the concentration effect of *M. anisopliae* was 0.596, 0.66, 0.282 and 1.137 in bioassay PTM 1st, 2nd, 3rd instar larvae, and pupae, respectively. However, pathogenicity of the different conidial concentration was similar. The parameters for the conditional time effect (γ_j) were in the order of $\gamma_1 < \gamma_2 < \gamma_3 > \gamma_4 > \gamma_5 > \gamma_6 < \gamma_7 > \gamma_8 < \gamma_9 > \gamma_{10}$ in the 1st instar larvae indicated that the maximum of mortality on day 3 after treatment with concentration of fungal. In the 2nd instar larvae, the order $\gamma_1 < \gamma_2 < \gamma_3 > \gamma_4 > \gamma_5 < \gamma_6 < \gamma_7 > \gamma_8 > \gamma_9 > \gamma_{10}$ indicated maximum mortality on day 3 after treatment with concentration of fungal. For the 3rd instar larvae, the order $\gamma_1 < \gamma_2 > \gamma_3 < \gamma_4 > \gamma_5 > \gamma_6 < \gamma_7 > \gamma_8 > \gamma_9 > \gamma_{10}$ indicated maximum mortality on day 4 after treatment with concentration of fungal and in the order $\gamma_1 = \gamma_2 = \gamma_3 < \gamma_4 < \gamma_5 > \gamma_6 < \gamma_7$ indicated maximum mortality on day 5 after treatment with concentration of fungal in the PTM pupae.

The estimates of τ_j for the cumulative time effect at a given dose d_i increased with time after inoculation, indicating cumulative mortality at a given dose d_i increased with time. The fitted parameter γ_j was different between the different bioassay methods, indicating that the time-specific effects and biocontrol potential of *M. anisopliae* at a specific conidial concentration also varied with the bioassay method.

3.4 Lethal time (LT_{50} and LT_{90}) of *M. anisopliae* against PTM larvae and pupae

The LT_{50} and LT_{90} values estimated by TCM modeling, all decreased with increasing fungal *M. anisopliae* conidial concentration for different stages and different instars of PTM larvae. The LT_{50} or LT_{90} could not be predicted for conidial concentrations, because of the mortality under them was less than 50% or 90%. Among the virulence to different stages of PTM showed the greatest virulence to the PTM larvae (2nd and 3rd instar), The lowest LT_{50} values were 1.01 d for 3rd instar larvae and 1.3 d for 2nd instar larvae at the higher conidial concentration of 2×10^8 conidia/mL. The lowest LT_{90} values were 2.6 d for 2nd instar larvae and 2.7 d for 3rd instar larvae, respectively. Compared with the virulence of the larvae. The fungal *M. anisopliae* has a longer infection time for pupae.

4. Discussion

The strains of Metarhizium and Beauveria are pathogenic to PTM larvae (Yaqiang et al., 2016; Yue et al., 2004; Yuan et al., 2016; Yuan et al., 2018) and PTM pupae (Zheng et al., 2019). Varma and Tandan (1996) showed that *M. anisopliae* was found to be pathogenic to six species of sugarcane pests (Varma & Tandan, 1996). There are many similar reported of entomopathogenic fungi infecting PTM larvae, The susceptibility of the PTM larvae to *M. anisoplae* decreased in the sequence of 2nd, 3rd, and 4th instar larvae at highest concentration (1.15×10^8 mL^{-1}) treatment the mortality was 96.67%, 90%, and 83.33% for 2nd, 3rd, and 4th instar larvae on 7th day, respectively (Zheng et al.,

2016). In our study, we found that *M. anisopliae* NGS71814 were virulent against to PTM larvae, with cumulative mortality reaching 95 ± 0.74% on day 4th on the 1st instar larvae, 100 ± 0.05% on day 3rd on the 2nd instar larvae and 100 ± 0.30% on day 5th on the 3rd instar larvae after inoculation at highest conidial concentration of 2.0×10^8 conidia/mL by using immersion method in bioassays. Kepenekci et al. (2013) showed that at 25℃ and 1000 IJs concentration, the larval mortality was 96 and 80% for *S. carpocapsae* and *H. bacteriophora*, respectively (Kepenekci et al., 2013). Instar larvae is one of the important factors affecting the pathogenicity of entomogenous fungi. There are some differences in the body wall structure and development degree of different larvae age. Therefore, there is a certain difference in the infectivity of fungal spores. In addition, insects have different sensitivities to different species of fungi in different larvae age, such as: *Plutella xylostella* larvae are more susceptible to infection by *Paecilomyces fuliginea* than 3rd and 4th instar larvae (Li et al., 2007). The 3rd and 4th instar larvae were more susceptible to infection by *B. bassiana* than the 2nd instar larvae (Vandenberg and Ramos, 1998). Our study results showed that the 2nd and 3rd instar PTM larvae were sensitivity to infested with *M. anisopliae* NGS71814 then 1st instar PTM larvae. In this study, we found that PTM larvae mortality was higher than Zheng et al., (2016) and Kepenekci et al., (2013) may be due to different virulence of the fungi species, different conidial concentration, including different inoculation methods in assay. Indicate that *M. anisopliae* NGS71814 is a potential biocontrol against PTM larvae stages. Therefore, the results of our study were consistent with previous findings of Yaqiang et al., (2016) and Kepenekci et al., (2013). However, although there is a difference between the sensitivity of the 1st, 2nd, and 3rd instar larvae to *M. anisopliae* NGS71814 or other entomogenous fungi, it is worthy further research.

The fungi of *M. anisopliae* is pathogenic to PTM pupae (Zheng et al., 2019), with significant commercial and industrial applications as a biopesticide (Pattemore et al., 2014). However, there are no similar reports of entomopathogenic fungi infecting PTM pupae (Zheng et al., 2019), although the PTM pupae stage is a potential target under field and storage conditions. Eivazian et al., (2018) showed that affects the interaction of *Steinernema carpocapsae* caused PTM pupae mortality reaching 89% (Eivazian et al., 2018). Zheng et al., (2019) conducted a study on the effects of *C. tenuipes* against PTM pupae by using four deference bioassays showed that the fungal infection caused 100%, 83.3%, 73.3%, and 85% mortality of PTM pupae in assays 1 - 4, respectively (Zheng et al., 2019). Our study found that at highest conidial concentration of 2.0×10^8 conidia/mL of *M. anisopliae* NGS71814 on the pupae mortality was 100% on day 5. The percentage mortality of pupae were significantly decreased by decreasing of conidial concentration was 95% on day 5 in 2.0×10^7 conidia/mL, 85.00%, 51.66%, 28.33% and 15.00%, respectively. It is possible that the conidia adhered more effectively to the cuticle of PTM pupae during immersion, compared with control (Tween 80; 0.05%) bioassays was 8.33% mortality.

Our results showed that lethal concentration of *M. anisopliae* conidia immersion of 1st, 2nd and 3rd instar PTM larvae were lower pupae. It is possible that the conidia adhered more effectively to the cuticle of PTM larvae during immersion, due to larvae cuticle were weakness then pupae shell. It is well known that the

entomopathogenic fungi penetrate primarily through the larvae cuticle and then enter the hemocoel, where they continue to grow and release metabolites resulting in mycosis and death. Beta-cypermethrin acts on the insect's central nervous system by blocking neuronal activity and causing rapid paralysis before death (Nian et al., 2015). When *M. anisopliae* was combined with sublethal doses of beta-cypermethrin, beta-cypermethrin was responsible for weakening the larvae, making them more sensitive to conidial penetration, at all concentrations, and *M. anisopliae* eventually kill the weakened larvae. Thus, PTM larvae more effects then pupae. This result is consistent with previous findings on effect of the entomopathogenic fungi *B. bassiana* on the various developmental stages of PTM (Zheng et al., 2016; Zheng et al., 2019).

The TCM modelling method is considered mathematically and biologically robust for evaluating data in time-concentration - response experiments because it incorporates the separate effects of the variables time and concentration simultaneously (Zheng et al., 2019). Many researchers of fungus-insect bioassays have been used this method (Lei & Feng, 2008; Ming & Xiao, 2005; Nian et al., 2015; Tian & Feng, 2006; Weibing et al., 2000; Xu & Feng, 2000; Zheng et al., 2016; Zheng et al., 2019; Zhou et al., 2016). In results showed that the fungal *M. anisopliae* NGS71814 to be effective against larvae and pupae, but their $LC_{50\ and}\ LT_{50}$ values determined by the fitted TCM relationships varied. The parameter β for the concentration effects were 0.596, 0.66, 0.282 and 1.137 in bioassay PTM 1st, 2nd, 3rd instar larvae, and pupae, respectively. which is lower than that for other chemical pesticides against insect pests (Tian & Feng, 2006; Shi et al., 2015; Zheng et al., 2019). This result is consistent with previous findings of Zheng et al., (2019) and Nian et al., (2015). This indicates that the pathogenicity efficiency of entomopathogenic fungi is weaker than that of chemical pesticides, which is in line with the general characteristics of microbial agents. Both conditional time effect (γ_j) and cumulative time effect (τ_j) changed with time, suggesting that conditional mortality and cumulative mortality are not only affected by time, but also by the concentration factor. Therefore, it is important to incorporate both time and concentration into the TCM model for simultaneous analysis.

5. Conclusion

In conclusion, our results demonstrated that virulence indices for strain NGS71814 of *M. anisopliae* could be against PTM larvae and pupae at high mortality, and it's also could be used as a biological control of PTM in the field and could be used in IPM programs. However, *M. anisopliae* showed the highest activity in all experiments. Despite the potential of entomopathogenic fungi in pest control, these bio-control agents have some disadvantages (sensitivity to environmental factors such as moisture, light and temperature), limiting their applications in storage and field conditions. However, new formulation technologies could improve in the further studies on the control efficiency of *M. anisopliae* on PTM in the field.

6. Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript

7. Reference

Anfora, G., Vitagliano, S., Larsson, M. C., Witzgall, P., Tasin, M., Germinara, G. S., & De Cristofaro, A. (2013). Disruption of *Phthorimaea operculella* (Lepidoptera: Gelechiidae) oviposition by the application

of host plant volatiles. *Pest Management Science*, *70*(4), 628–635.

Eivazian, K. N., Sanatipour, Z., Mohammadi, D., & Koppenh Fer, A. M. (2018). Developmental stage affects the interaction of *steinernema carpocapsae*, and abamectin for the control of *Phthorimaea operculella*, (lepidoptera, gelechidae). Biol. Control, S1049964418301968.

Gilboa, S., & Podoler, H. (1995). Presence-absence sequential sampling for potato tuber worm (Lepidoptera: gelechiidae) on processing tomatoes: selection of sample sites according to predictable seasonal trends. *Journal of Economic Entomology*. *88*(5), 1332-1336.

Graf, J. E. (1917). The potato tuber moth. U.S. Department of Agriculture Bulletin. 427.

Kepenekci, I., Tulek, A., Alkan, M., & Hazir, S. (2013). Biological control potential of native entomopathogenic nematodes against the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in Turkey. *Pakistan Journal of Zoology*. *45*(5).

Kikuchi, H., Hoshi, T., Kitayama, M., Sekiya, M., Katou, Y., Ueda, K., Kubohara, Y., Sato, H., Shimazu, M., Kurata, Sh., & Oshima, Y. (2009). New diterpene pyrone-type compounds, Metarhizins A and B, isolated from entomopathogenic fungus, *Metarhizium flavoviride* and their inhibitory effects on cellular proliferation. *Tetrahedron*. *65*(2), 469-477.

Li-Hua, L. V., Yu-Rong, H. E., Ya-Jing, W. U., Xia, F., & Huan-Yukg, C. (2007). The time-dose-mortality model of a *Paecilomyces fumosoroseus* isolate on the diamondback moth, *Plutella xylostella*. Acta Entomologica Sinica.

Liu, H., Skinner, M., Parker, B. L., & Brownbridge, M. (2002). Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (deuteromycotina: hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (hemiptera: miridae). Journal of Economic Entomology. *95*(4), 675-681.

Liu, Y., Hu, C. H., Wang, C. Y., Xiong, Y., Li, Z. K., & Xiao, C. (2018). Occurrence of parthenogenesis in potato tuber moth: *Journal of Insect Science*. *18*(1).

Feng, M. G., & Pu, X. Y. (2005). Time–concentration–mortality modeling of the synergistic interaction of Beauveria bassiana and imidacloprid against Nilaparvata lugens. *Pest Management Science: Formerly Pesticide Science*, *61*(4), 363-370.

Moore, D., Reed, M., Patourel, G. L., Abraham, Y. J., & Prior, C. (1992). Reduction of feeding by the desert locust, schistocerca gregaria, after infection with *Metarhizium flavoviride*. Journal of Invertebrate Pathology. *60*(3), 0-307.

Nian, Xiao-ge., He, Yu-rong., Lu, Li-hua., & Zhao, R. (2015). Evaluation of the time–concentration– mortality responses of *Plutella xylostella* larvae to the interaction of *Isaria fumosorosea* with the insecticides Beta-cypermethrin and *Bacillus thuringiensis*. *Pest Management Science*. *71*(2), 216-224.

Pattemore, J. A., Hane, J. K., Williams, A. H., & Wilson, A. B. (2014). The genome sequence of the biocontrol fungus *Metarhizium anisopliae* and comparative genomics of Metarhizium species. BMC Genomics. *15*(1).

Robertson, J. L., & Preisler, H. K. (1992). Pesticide bioassays with arthropods. Taylor Print on Dema.

Rondon, S. I. (2010). The potato tuber worm: a literature review of its biology, ecology, and control. American Journal of Potato Research, 87(2), 149-166.

Rondon, S. I., Debano, S. J., Clough, G. H., Dogramaci, M., & Barbour, J. (2007). Biology and management of the potato tuber worm in the pacific northwest. [Covallis, Or.]: Oregon State University Extension Service.

Sabbour M. M., & Singer, S. M. (2015). Evaluations of Two *Metarhizium* varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt, International Journal of Science and Research.

Sabbour M. M. (2015). The Toxicity Effect of Nano Fungi *Isaria fumosorosea* and *Metarhizium flavoviride* against the Potato Tuber Moth, *Phthorimaea operculella* (Zeller). American Journal of Biology and Life Sciences, *3*(5):155-160.

Shi, Y. W., Qin, X., Wang, Z., Wang, M., Liu, J., Chen, Y., Zhang, R. J., & Liu, Y. D. (2015). Analysis of the Time–Concentration–Mortality Response of *Nilaparvata lugens* (Hemiptera: Delphacidae) to Paichongding. Journal of Economic Entomology, tov179.

Sporleder, M., Kroschel, J., Quispe, M. R. G., & Lagnaoui, A. (2004). A temperature-based simulation model for the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; gelechiidae). *Environmental Entomology*. *33*(3), 477-486.

Tang, Q. Y., & Feng, M. G. (2010). DPS data processing system-experimental design, statistical analysis and data mining. Science, Beijing.

Thomsen, L., & Eilenberg, J. (2000). Time-concentration mortality of *Pieris brassicae* (lepidoptera: pieridae) and *Agrotis segetum* (lepidoptera: noctuidae) larvae from different destruxins. Environmental Entomology, 29(5), 1041-1047.

Tian, L., & Feng, M. G. (2006). Evaluation of the time-concentration-mortality responses of *Plutella xylostella* larvae to the interaction of *Beauveria bassiana* with a nereistoxin analogue insecticide. *Pest Management Science*. *62*(1), 69–76.

Vandenberg, J. D., Ramos, M., & Altre, J. A. (1998). Dose-response and age- and temperature-related susceptibility of the diamondback moth (lepidoptera: plutellidae) to two isolates of *Beauveria bassiana* (hyphomycetes: moniliaceae). *Environmental Entomology*. *27*(4), 1017-1021.

Varma, A., & Tandan, B. K. (1996). Pathogenicity of three entomogenous fungi against insect pests of sugarcane. *Journal of Biological Control*. *10*(1&2).

Weibing, S., Lei, Z., & Mingguang, F. (2008). Time-concentration-mortality responses of carmine spider mite (acari: tetranychidae) females to three hypocrealean fungi as biocontrol agents. *Biological Control*. *46*(3), 495-501.

Xu, J. H., & Feng, M. G. (2000). The time–dose–mortality modeling and virulence indices for two entomophthoralean Species, *Pandora delphacis* and *P. neoaphidis*, against the Green Peach

Yaqiang, Z., Shengjie, S., Bin, C., Guanli, X., Lei, Y., Chunping, L., & Zhengyue, L.

(2016). The infection effect of *Metarhizium anisopliae* kma0107 against the larvae of potato tuber moth, *Phthorimaea operculella* (zeller). Journal of Yunnan Agricultural University (Natural Science).

Yuan, H., Lei, Z., Rondon, S. I., & Gao, Y. (2016). Potential of a strain of *Beauveria bassiana* (hypocreales: cordycipitaceae) for the control of the potato tuberworm, *Phthorimaea operculella* (zeller). *International Journal of Pest Management*. *63*(4), 1-3.

Yuan, H. G., Wu, S. Y., Lei, Z. R., Rondon, S. I., & Gao, Y. L. (2018). Sub-lethal effects of, *Beauveria bassiana*, (balsamo) on field populations of the potato tuberworm, *Phthorimaea operculella* zeller in China. Journal of Integrative Agriculture, 17(4), 911-918.

Yue-Xian, S., Zheng-Yue, L. I., Fu-Rong, G., Nai-Sheng, Y., CHEN-Bin, & Ruo-Qing, X. U. (2004). Susceptiblity of *Phthorimaea operculella* (zeller) larvae to 8 isolates of Beauveria. Southwest China Journal of Agricultural Sciences.

Zheng, Y., He, S., Chen, B., Su, Z., Wang, W., Zhang, L., & Xiao, G. (2019). Time-concentration-mortality response of potato tuber moth pupae to the biocontrol agent *Cordyceps tenuipes*. Biocontrol Science and Technology, 1–14.

Zhou, X., Su, X., & Liu, H. (2016). A floatable formulation and laboratory bioassay of *Pandora delphacis* (Entomophthoromycota: Entomophthorales) for the control of rice pest *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). Pest Management Science. *72*(1), 150–154.

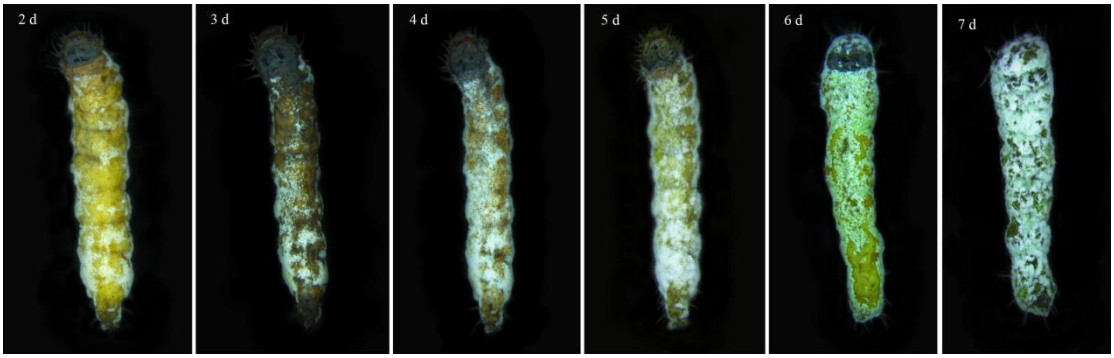

Figure. 1. Symptoms of the infected PTM larvae by *M. anisopliae* in 7 days after the inoculation.

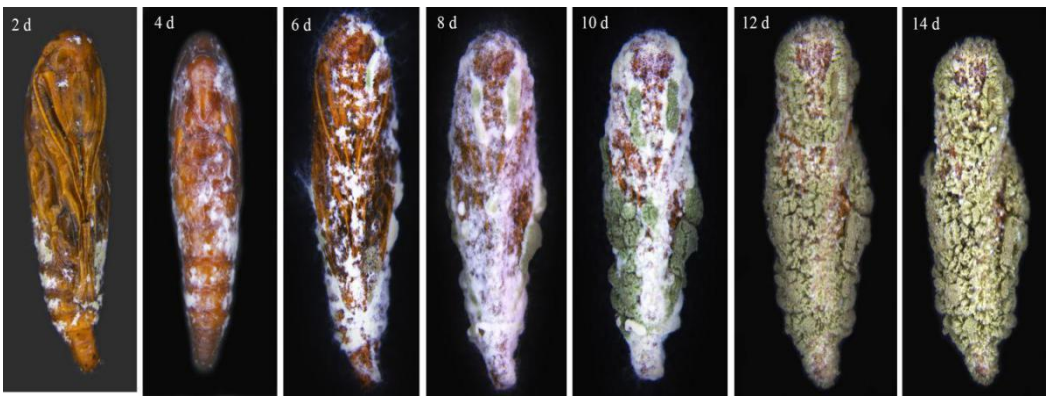

Figure. 2. Symptoms of *M. anisopliae* infection on the PTM pupae within 14 days after inoculation.

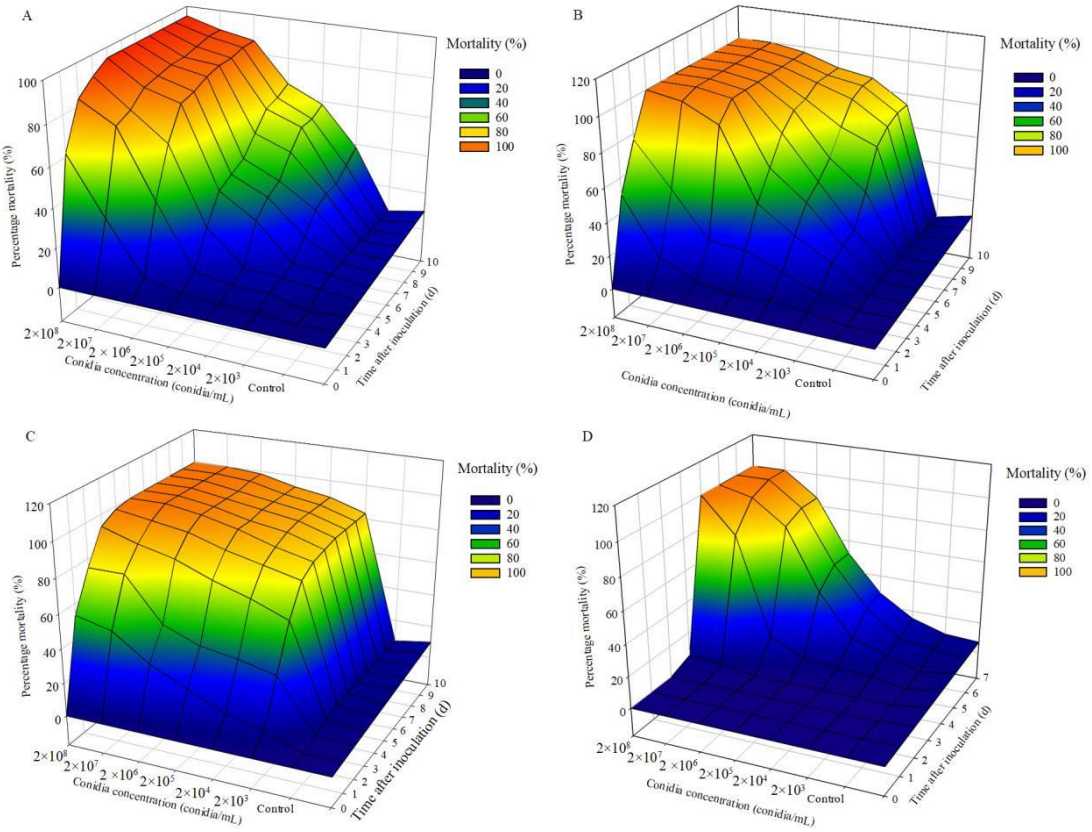

Figure. 3. Mortality of *P. operculella* larvae and pupae inoculated with *M. anisopliae* using 6 different conidial suspension concentration of fungal. (A) Mortality of the 1st instar PTM larvae after the infection of *M. anisopliea.* (B) Mortality of the 2nd instar PTM larvae after the infection of *M. anisopliea.* (C) Mortality of the 3rd instar PTM larvae after the infection of *M. anisopliea.* (D) Mortality of the pupae PTM after the infection of *M. anisopliea*.