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## Application of *Pandora neoaphidis* for the control of aphid pests in vegetable crops in Thailand

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**Abstract** The entomopathogenic fungus *Pandora neoaphidis* has great potential for use in the biological control of aphids. In the present study, aphid cadavers were collected from three provinces in Northern Thailand: Chiang Rai, Chiang Mai, and Lamphun. Pathogenicity of three aphid species, *Myzus persicae*, *Lipaphis erysimi*, and *Aphis fabae*, was evaluated in the laboratory. The pure culture of PAN04 showed the highest pathogenicity for the respective aphid species, with average of  $95.56\% \pm 3.25\%$ ,  $91.83\% \pm 2.18\%$ , and  $92.36\% \pm 2.73\%$ , respectively. Additionally, the lethal concentration (LC50) based on laboratory tests was calculated for each aphid species, which were 75.55, 78.44, and 59.78 spores/mm<sup>2</sup>, respectively. Under greenhouse conditions, *P. neoaphidis* suppressed aphid populations with a percent cumulative mortality (PCM) of  $50.94\% \pm 16.55\%$ ,  $45.17\% \pm 4.27\%$ , and  $47.36\% \pm 11.56\%$ , while the mean time to death (MTD) was  $5.67 \pm 1.15$ ,  $5.56 \pm 1.53$ , and  $3.67 \pm 0.58$  days, respectively. In field conditions, the PCM of the respective aphid species averaged  $45.00\% \pm 80\%$ ,  $34.21\% \pm 15.3\%$ , and  $34.82\% \pm 7.24\%$ , while the MTD was  $6.00 \pm 2.00$ ,  $6.67 \pm 0.58$ , and  $7.42 \pm 0.58$  days. According to the present study, *P. neoaphidis* has great potential for controlling economically important aphid species in Thailand.

**Keywords:** *Pandora neoaphidis*, Aphids, Pathogenic fungus, Biocontrol

### Introduction

Aphids (Hemiptera: Aphididae) are key pests of food crops, causing significant yield losses ranging from 12.79% to 61.07% (Owain *et al.*, 2008). Various species of aphids have been identified in Thailand, including the cabbage aphid (*Lipaphis erysimi*), green peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*), soybean aphid (*Aphis craccivora*), and long beans aphid (*Aphis fabae*). The use of foliar insecticides is a common method to control aphid infestations, but it can lead to human toxicity due to residues in food and the environment. Additionally, insecticides can harm natural enemies and

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disrupt biological control of aphids. The development of resistance in aphids to insecticides is a growing concern due to frequent applications and overuse (Department of Agriculture, 2011; Stufkens *et al.*, 2005; Owain *et al.*, 2008).

Pathogenic fungi have been used as biocontrol agents to reduce their reliance on pesticides (DeBach and Rosen, 1991). *Pandora neoaphidis* is an important fungal pathogen that specifically targets aphids (Wilding and Brady, 1984). This fungal pathogen has been found to infect various aphid species, including cotton, spinach, cereal, and tobacco aphids (McLeod *et al.*, 1998; David *et al.*, 2003; Wilding *et al.*, 1990; Surendra and Semtner, 2006). Studies have shown that *P. neoaphidis* can effectively reduce tobacco aphid populations by 90% (Elkassabany *et al.*, 1992) and control aphid dispersion by 33-37% for up to two weeks after treatment in both glasshouses and open fields (Surendra and Semtner, 2006). Sheng (2010) identified *P. neoaphidis* as an entomopathogenic fungus against turnip aphids and demonstrated pathogenicity at a concentration of 18.2 conidia/mm<sup>3</sup>. The advantage of using fungal pathogens for biocontrol is their compatibility with environmental and health regulations. In Thailand, *Pandora neoaphidis* is a local fungus found in kale, cabbage, bok choy, chili, eggplant, yardlong bean, and cowpea crop fields. However, research on its pathogenicity is limited. Therefore, this study aimed to isolate pure cultures of *P. neoaphidis* collected from crop fields in three provinces in northern Thailand, and evaluated the concentration required to cause 50% mortality in aphid populations.

## **Materials and methods**

### ***Sample collection and isolation of pure culture***

Infected aphids were collected from three provinces in northern Thailand: Chiang Rai, Chiang Mai, and Lamphun. The latitude and longitude ranges for these regions were 18°46.2' N to 19°4'11' N and 99°49'9.0' E to 100°11'34' E. Field crops such as kale, cabbage, bok choy, chili, eggplant, yardlong beans, and cowpeas infested with aphids were randomly sampled for fungal infection. The sampling area was 400 sqm per plot, with six plots per province (totaling 18 plots). Aphid cadavers showing symptoms of fungal infection were observed on the leaves, with approximately 50 leaves per plant and 100 plants per plot. Collection was done 1-2 times per month, totaling 12 times per year, between September 2014 and 2015 (Dent and Walton, 1997). The samples were placed in plastic boxes (18 cm × 18 cm × 12 cm) under cooling conditions and transported to the laboratory (MJU Biological Control Laboratory; MJU-BCL) for species classification. *P. neoaphidis* was isolated from the collected aphid

cadavers using the dilution plate technique and cultivated on potato dextrose agar (PDA), Sabouraud dextrose agar (SDA), and Sabouraud dextrose agar supplemented with yeast extract (SDAY) at  $25\pm 2^{\circ}\text{C}$  and  $70\pm 10\%$  RH under a 12:12 h light-dark cycle. Morphology was examined under a compound microscope at a magnification settings of 10X-40X (three replicates per isolate). The characteristics of hyphae on insect bodies were observed for the formation, color, and shape of the spores. Optimized enrichment for all isolates was performed following the protocol described by Rungkiat and Samaporn (2014).

***Substance suspended at 50% of aphids will be expected to die (lethal concentration; LC<sub>50</sub>) of laboratory tests***

The pathogenicity of each isolate was investigated in different aphid species, namely, *Lipaphis erysimi*, *Myzus persicae*, and *Rhopalosiphum maidis*. Spore suspensions were prepared from *P. neoaphidis* agar culture with 0.02% Tween 80 in sterile distilled water. Spores were counted using a Neybauer Hemocytometer and the suspension concentration was adjusted to  $10^{10}$  spores/mm<sup>3</sup> (Poinar and Thomas, 1984). Spore suspensions were sprayed on leaves infested with aphids (50 aphids per leaf). The samples were maintained at  $20\pm 2^{\circ}\text{C}$  and 80% RH in the dark. The number of aphid cadavers was monitored every 24 h for 120 h, and percent cumulative mortality (PCM) was calculated using Abbott's Correction Formula (Abbott, 1925). The suspension concentration that was lethal to 50% of the aphid population was also investigated at concentrations of 0, 10, 50, 100, 150, 200, 250, 300, and 350 spores/mm<sup>3</sup> following the method of Poinar and Thomas. Aphid cadavers were recorded for PCM calculations at each concentration. The lethal concentration to 50% of the aphid population at different concentrations was calculated using Finney's probit analysis method, and the values were determined using regression analysis (Finney, 1971). A completely randomized design (CRD) with five replications was used in the experimental design.

***Pathogenicity test under greenhouse and field condition***

Plants targeted by three aphid species, chili, kale, cabbage, and yard-long beans, were planted in plots (30 cm in diameter), with a total of 30 plots per treatment. After planting for 24 h, leaves infested with aphids were placed in the plot samples with 20 aphids per plot and reproduced under greenhouse and field conditions. The treatment solution was compared with 1) a *P. neoaphidis* suspension concentration lethal to 50%, 2) 0.02% Tween 80

surfactant in sterile distilled water, and 3) dimethoate (15-20 cc/20 L) + 0.02% Tween 80 surfactant. The treatment was sprayed on plant samples every 7 d for 8 weeks, and infected aphids were monitored every 24 h on 10 plants per treatment for percent cumulative mortality (PCM) and mean time to death (MTD) calculation. This experiment was conducted using a completely randomized design (CRD) with 10 replicates. The field conditions were similar to those under greenhouse conditions, with an experimental plot size of 50×500 cm in a CRD experimental design with three replications (Figure 1).



**Figure 1.** This experiment of pathogenicity of *P. neoaphidis* fungi test under greenhouse and field condition A. pathogenicity tests fungi to *M. persicae* in chili crop, B. *L. erysimi* in cabbage crop and C. *R. maidis* in yard long beans crop

### ***Data analysis***

Abbott's formula is a simple correction for percent cumulative mortality. If  $x(t)$  represents the percentage of the population that consists of infected aphids and  $x(c)$  represents the percentage of the population that consists of non-infected aphids, then the corrected mortality can be calculated as  $[x(c) - x(t)] / x(c)$ . The mean Time to Death (MTD) involved recording the time of death for individual aphids infected with pathogenic fungi and calculating the time difference between the time of infection and the time of death for each case. To calculate the mean, we add up all the survival durations and divide the total by the number of cases, resulting in the mean death time. The mortality data were then analyzed using regression probit analysis to

determine the Lethal Time (LT<sub>50</sub>). The collected data were subjected to an analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test (DMRT) at a significance level of  $P \leq 0.05$ .

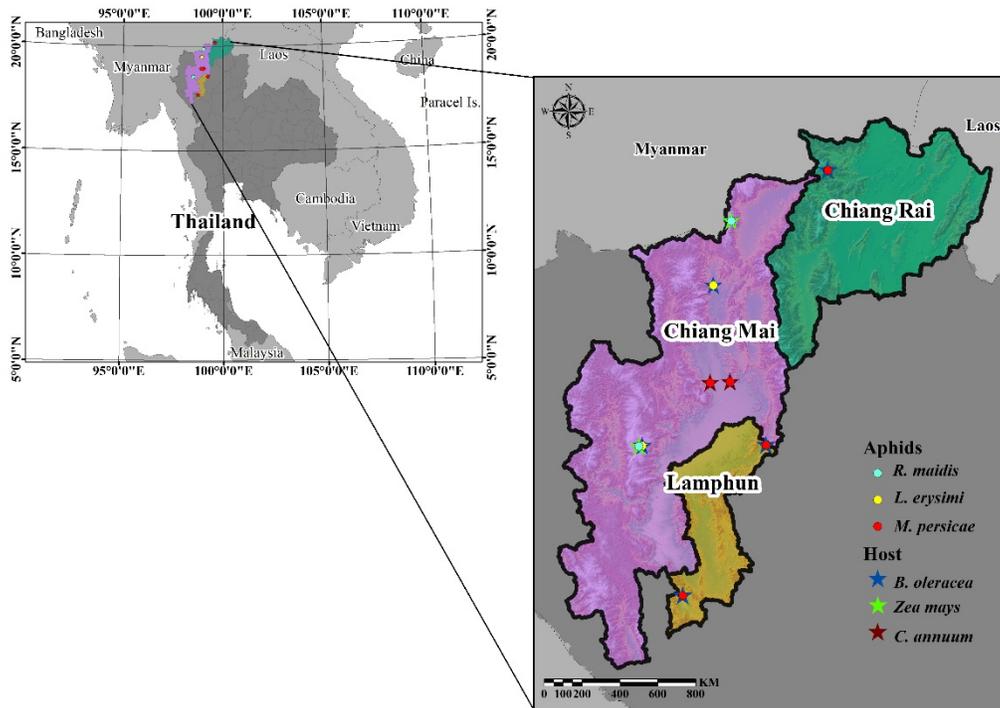
## Results

### *Classification of aphids and isolation of pure culture*

A total, 716 aphids were collected from the northern provinces of Thailand, including Chiang Rai, Chiang Mai, and Lamphun. The field experiment covered 20 locations with 19 target plants, including kale, Chinese cabbage, bok choy, cabbage, broccoli, bird's eye chili, spur chili, bell peppers, tomatoes, eggplant, green brinjal, potatoes, yardlong beans, and flowering plants, such as marigolds and chrysanthemums. Field crops such as corn and soybeans as well as fruit trees such as oranges and lemons were also included.

Individual aphids were identified as belonging to three species: *M. persicae*, *L. erysimi*, and *A. fabae*, with 246, 428, and 42 aphids, respectively. Classification of the aphids based on their host plant specificity revealed that they were major pests of *Capsicum annuum*, *Brassica oleracea*, and *Zea mays* crops, with 268, 406, and 42 aphids, respectively. The results of aphid collection infected with *P. neoaphidis* in the three provinces are presented in Figure 2.

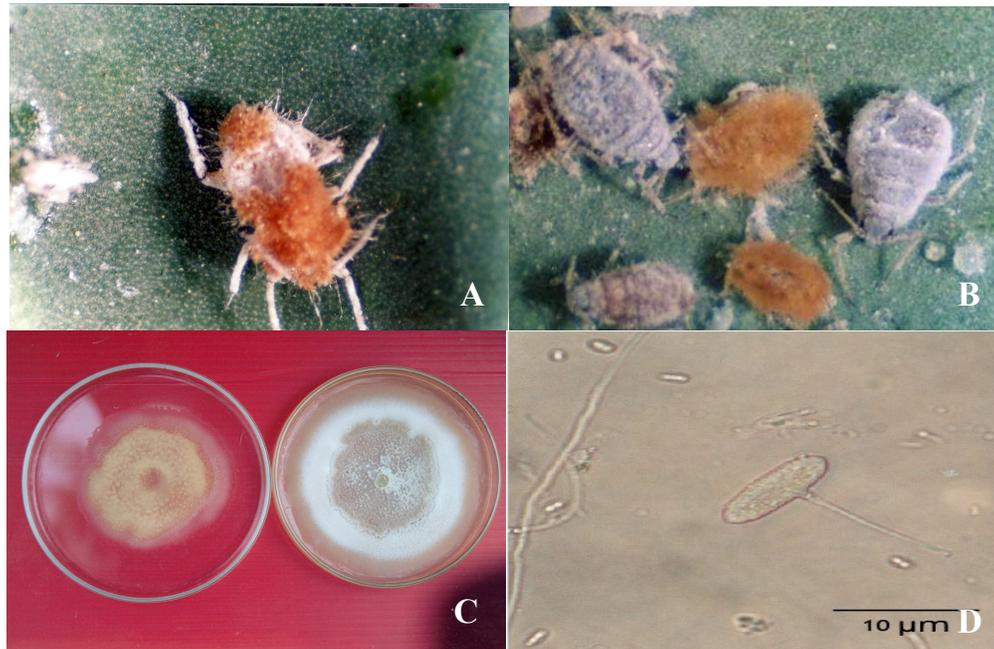
The fungal pathogen (*P. neoaphidis*) is specific to aphids and showed a continuous increase from September 2014 to March 2015. It is characterized by hyphae on insect bodies, clear spores, and a variety of shapes, with different round sizes measuring 8-10 micrometers (Figure 3). The prevalence of fungal pathogens and infected aphids peaked at 120% and 82.16%, respectively, and then steadily declined from April to September 2021 (Figure 4). The fungal pathogen was isolated from biological samples, specifically aphid cadavers displaying symptoms of fungal infection, using the method described by Rungkiat and Samaporn (2014). The data showed a range of 36.07-95.12% or an average monthly value of  $56.94 \pm 20.51\%$ . The number of isolates and purification of fungal pathogens increased from November 2020 to February 2021, reaching 73.68-95.12%. The PAN04 isolate demonstrated higher infection efficiency against *L. erysimi*, *A. fabae*, and *M. persicae* species, with infection rates of  $91.80 \pm 2.18$ ,  $92.36 \pm 2.73$ , and  $95.56 \pm 3.25$ , respectively.



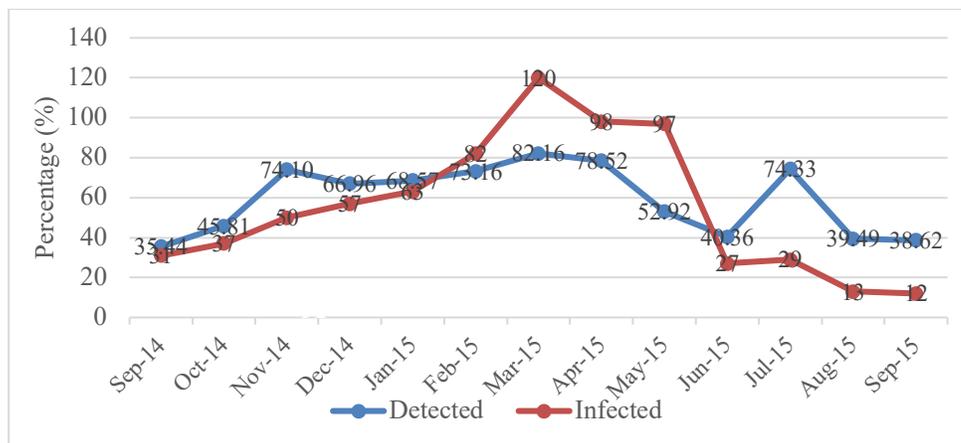
**Figure 2.** Aphids were infected by *P. neoaphidis* in the areas of Chiang Rai, Chiang Mai, and Lamphun

### ***Lethal concentration 50 (LT<sub>50</sub>) estimates***

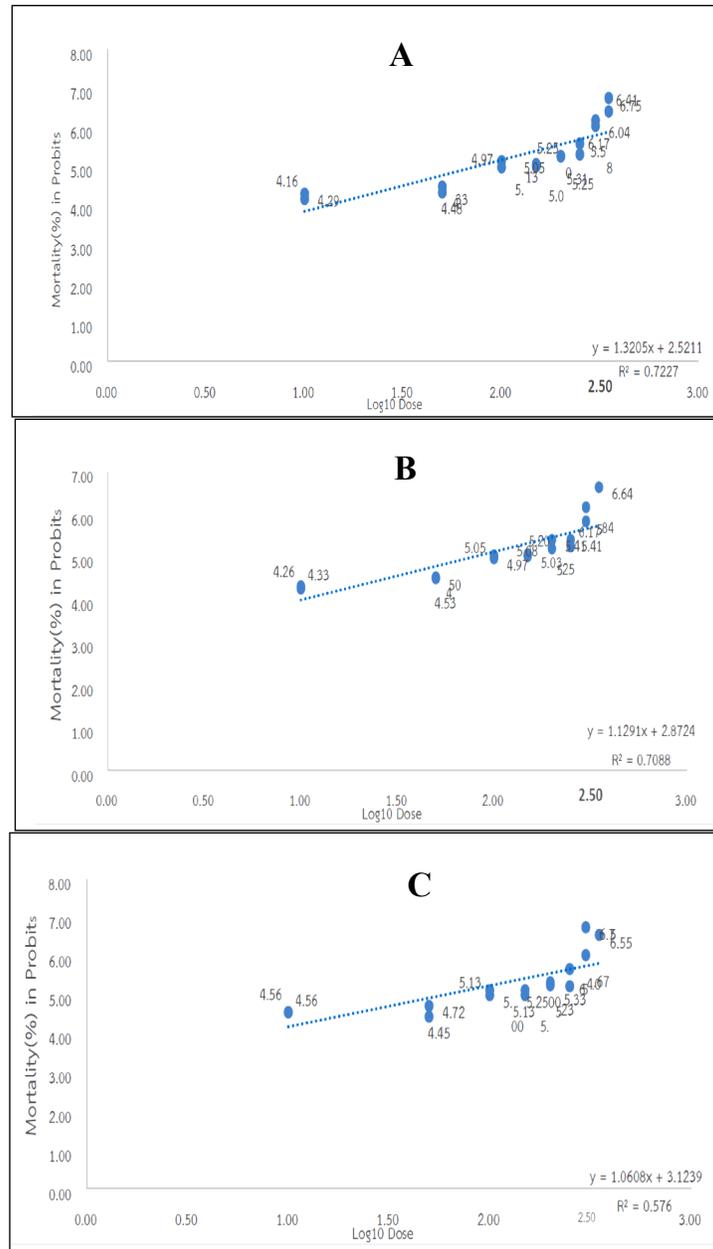
The lethal concentration 50 (LT<sub>50</sub>) estimates for *M. persicae* infected with *P. neoaphidis* are 75.55 spores/mm<sup>3</sup>. Linear regression analysis showed a graph slope value of 1.321, secant points of 2.479, standard deviation (S.D) of 0.757, and regression (R<sup>2</sup>) value of 0.723. For *L. erysimi*, the LT<sub>50</sub> was 78.44 spores/mm<sup>3</sup>, with a slope value of 1.12, secant points of 2.12, an S.D. of 0.88, and R<sup>2</sup> of 0.709. *A. fabae* had an LT<sub>50</sub> of 59.78 spores/mm<sup>3</sup>, slope value of 1.06, secant point of 1.87, S.D. of 0.94, and R<sup>2</sup> of 0.57 (Figure 5).



**Figure 3.** Characteristics of aphids destroyed by A, B. *P. neoaphidis* C. *P. neoaphidis* isolates exhibiting colony morphology on SDAY medium, and D. spore



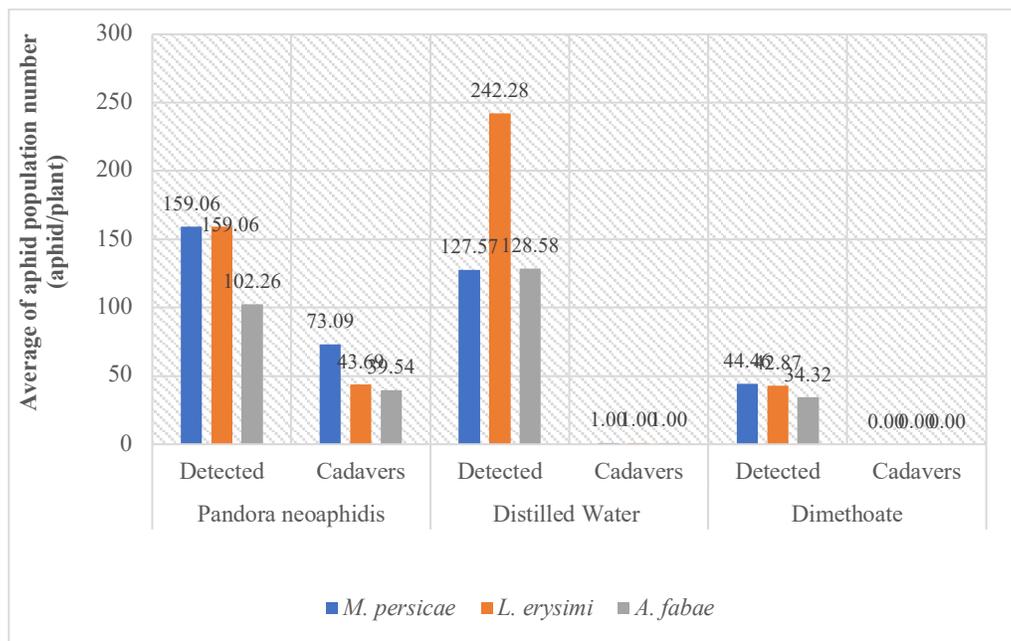
**Figure 4.** The variation of *P. neoaphidis* and infected aphid population in September 2020-2021



**Figure 5.** Linear regression analysis of A. *M. persicae*, B. *L. erysimi*, and C. *R. maidis* infected with *P. neoaphidis*

**Pathogenicity test under greenhouse and field condition**

The pathogenicity of *P. neoaphidis* in different aphid species under greenhouse conditions was compared using distilled water and insecticide (dimethoate). The aphid population in all treatments increased continuously throughout the 8-week experiment. However, the total number of aphid cadavers increased significantly in the *P. neoaphidis* suspension treatment, indicating lower aphid survival than in the distilled water treatment. The number of aphid cadavers per leaf was 73.09, 43.69, and 39.54 for *M. persicae*, *L. erysimi*, and *A. fabae*, respectively, at week 8 after the suspension treatment, whereas the distilled water and insecticide (dimethoate) treatments had 1 and 0 aphids per leaf, respectively (Figure 6).



**Figure 6.** Number of survival and cadavers of *M. persicae*, *L. erysimi*, and *A. fabae* in different treatment (*P. neoaphidis* suspensions, distilled water and insecticide dimethoate) under greenhouse condition at week 8

The percentage of cumulative mortality (PCM) in *P. neoaphidis* suspension treatment was  $50.94\% \pm 16.55\%$  for *M. persicae*, which was higher than that of the distilled water treatment at  $21.99\% \pm 6.21\%$  and lower than that of the insecticide (dimethoate) treatment at  $85.05\% \pm 5.33\%$ . The mean time to death (MTD) was  $5.67 \pm 1.15$  days for suspensions,  $17 \pm 1.73$  days for distilled

water, and 1.00 day for dimethoate treatment. For *L. erysimi*, the PCM was 45.17% ± 4.27% for suspensions, 29.51% ± 2.38% for distilled water, and 92.10% ± 9.18% for dimethoate treatment. The MTD was 5.56 ± 1.53 days for suspensions, 17.00 ± 4.25 days for distilled water, and 1.00 day for dimethoate treatment. For *A. fabae*, the PCM was 47.36% ± 11.56% for suspensions, 22.17% ± 7.96% for distilled water, and 80.24% ± 7.59% for dimethoate treatment. The MTD was 3.67 ± 0.58 days for suspensions, 14.33 ± 4.04 days for distilled water, and 1.00 day for dimethoate treatment (Table 1, 2).

**Table 1.** The percent cumulative mortality (PCM) of different aphid species in *P. neoaphidis* suspensions, distilled water, and insecticide (dimethoate) treatment under greenhouse condition at week 8

Treatment	Percent. Cumulative mortality - PCM		
	<i>M. persicae</i>	<i>L. erysimi</i>	<i>A. fabae</i>
<i>P. neoaphidis</i>	50.94 ± 16.55a	45.17 ± 4.27a	47.36 ± 11.56a
Distilled Water	21.99 ± 6.21b	29.51 ± 2.38b	22.17 ± 7.96b
Dimethoate	85.10 ± 5.33c	92.10 ± 9.18c	80.24 ± 7.59c

significant differences at  $p \leq 0.05$  and  $p \leq 0.01$  respectively according to DMRT.

<sup>1/</sup> Means followed by the same small letter with the same column of cultivar are not significant different

**Table 2.** The mean time to death (MTD) of different aphid species in *P. neoaphidis* suspensions, distilled water, and insecticide (dimethoate) treatment under greenhouse condition at week 8

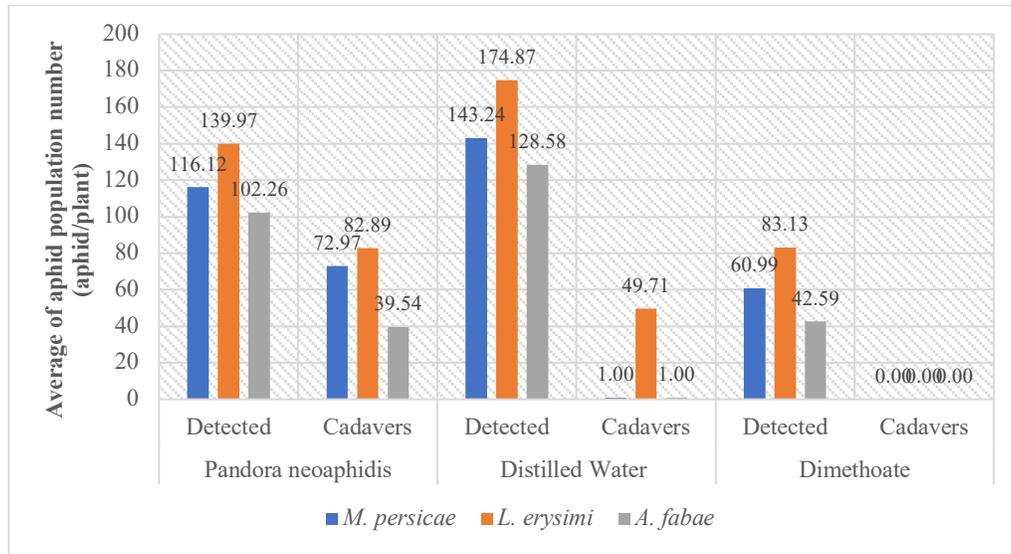
Treatment	Mean Time to Death -. MTD		
	<i>M. persicae</i>	<i>L. erysimi</i>	<i>A. fabae</i>
<i>P. neoaphidis</i>	5.67 ± 1.15a <sup>1</sup>	5.56 ± 1.53a	3.67 ± 0.58a
Distilled Water	17.00 ± 1.73b	17.00 ± 4.25b	14.33 ± 4.04b
Dimethoate	1.00 ± 0.00c	1.00 ± 0.00c	1.00 ± 0.00c

significant differences at  $p \leq 0.05$  and  $p \leq 0.01$  respectively according to DMRT.

<sup>1/</sup> Means followed by the same small letter with the same column of cultivar are not significant different

The pathogenicity of *P. neoaphidis* under field conditions showed a similar trend to that observed under greenhouse conditions. The number of aphid cadavers exhibiting symptoms of fungal infection in the suspension treatment was 72.97 aphids per leaf for *M. persicae*, which was higher than the number of 1 aphid per leaf exhibiting symptoms of fungal infection in the insecticide (dimethoate) treatment. The percent cumulative mortality (PCM)

was 45.80%, 72.26±25.34%, and 3.00±2.00%, and the mean time to death (MTD) was 6.00±2.00, 22±3.46, and 1.00 days in the *P. neoaphidis* suspensions, distilled water, and insecticide (dimethoate) treatments, respectively. The number of aphid cadavers of *L. erysimi* was 82.89 and 49.71 aphids per leaf in the suspensions and distilled water treatments, respectively, and was not detected in the insecticide (dimethoate) treatment. The PCM was 34.21±15.37%, 85.12±10.16%, and 17.86±8.37%, and the MTD was 6.67±0.58, 21.33±3.51, and 1.00 days in the suspensions, distilled water, and dimethoate treatments, respectively. The number of aphid cadavers of *A. fabae* was 39.54 aphids per leaf in the suspension treatment and 1 aphid per leaf in the distilled water treatment, whereas it was not detected in the insecticide (dimethoate) treatment. The PCM was 34.82±7.24%, 78.66±8.24%, and 7.76±3.72%, and the MTD was 7.42±0.58, 19.00±1.00, and 1.00 days in the suspensions, distilled water, and dimethoate treatments, respectively (Figure 7 and Tables 3, 4).



**Figure 7.** Number of survival and cadavers of *L. erysimi*, *M. persicae* and *A. fabae* in different treatment (*P. neoaphidis* suspensions, distilled water, and insecticide dimethoate) field condition at week 8

**Table 3.** The percent cumulative mortality (PCM) of different aphid species in *P. neoaphidis* suspensions, distilled water, and insecticide (dimethoate) treatment under field condition at week 8

Treatment	Percent. Cumulative mortality - PCM		
	<i>M. persicae</i>	<i>L. erysimi</i>	<i>A. fabae</i>
<i>P. neoaphidis</i>	45.00 ±8.00a	34.21± 15.37a	34.18 ±11.47a
Distilled Water	3.00 ±2.00b	17.86 ±8.37b	7.24 ±3.72b
Dimethoate	72.66 ±25.34c	85.82 ±10.18c	78.76 ±2.24c

significant differences at  $p \leq 0.05$  and  $p \leq 0.01$  respectively according to DMRT.

<sup>1/</sup> Means followed by the same small letter with the same column of cultivar are not significant different

**Table 4.** The mean time to death (MTD) of different aphid species in *P. neoaphidis* suspensions, distilled water, and insecticide (dimethoate) treatment under field condition at week 8

Treatment	Mean Time to Death -. MTD		
	<i>M. persicae</i>	<i>L. erysimi</i>	<i>A. fabae</i>
<i>P. neoaphidis</i>	6.00 ± 2.00a	6.67 ±0.58a	7.42 ±0.58a
Distilled Water	22.00 ±3.46b	21.33 ±3.51b	19.00 ±1.00b
Dimethoate	1.00 ± 0.00c	1.00 ±0.00c	1.00 ±0.00c

significant differences at  $p \leq 0.05$  and  $p \leq 0.01$  respectively according to DMRT.

<sup>1/</sup> Means followed by the same small letter with the same column of cultivar are not significant different

## Discussion

The populations of pathogenic fungi showed variability throughout the year and were highly prevalent in February-March, which provided favorable conditions for their survival. The pathogenic characteristics were influenced by density-dependent factors. The fungus *P. neoaphidis* is specific to aphids, such as *M. persicae*, *L. erysimi*, and *A. fabae*, which are major pests of *Brassica oleracea*, *Capsicum annum*, and *Zea mays*. The percentage of infected aphids ranged from 11.13% to 91.68%. Temperature and host range affect the growth and severity of fungi, leading to insect diseases (Tymon *et al.*, 2004; Yeo *et al.*,

2003). Previous studies have shown that the entomopathogenic fungus *P. neoaphidis* significantly reduces aphid populations (McLeod *et al.*, 1998; Pell *et al.*, 2001), causing natural epizootics in aphid populations of up to 71 species (Chen *et al.*, 2007; Wilding and Brady, 1984). Therefore, *P. neoaphidis* is a widespread and important specialist fungal pathogen of aphids and should be promoted as a biocontrol agent to reduce the widespread use of chemical pesticides in Thailand.

The isolation of pathogenic fungi in pure culture using the method described by Rungkiat and Samaporn (2014) resulted in an efficiency of  $56.94 \pm 20.51\%$ . The PAN04 isolate exhibited higher efficiency, with lethal concentration 50 (LT<sub>50</sub>) estimates of 75.55, 78.44, and 59.78 spores/mm<sup>3</sup> for *M. persicae*, *L. erysimi*, and *A. fabae* species, respectively. The differentiation of fungal pathogen fragments led to LT<sub>50</sub> values. Xu and Feng (2001) found that fiber suspensions showed LT<sub>50</sub> values ranging from 22.8 to 162 fiber/mm<sup>3</sup>, while spore suspensions ranged from 4.7 to 49.4 spores/mm<sup>3</sup>.

The percent cumulative mortality (PCM) under greenhouse conditions varied significantly between the treatments. The *P. neoaphidis* suspensions treatment showed higher PCM values compared to the distilled water treatment, with  $50.94 \pm 16.55\%$ ,  $45.17 \pm 4.27\%$ , and  $47.36 \pm 11.56\%$  for *M. persicae*, *L. erysimi*, and *A. fabae*, respectively. The mean time to death (MTD) of the suspensions treatment caused aphids to die more rapidly than the distilled water treatment, with results of  $5.67 \pm 1.15$ ,  $5.56 \pm 1.53$ , and  $3.67 \pm 0.58$  days, respectively.

Insecticide (dimethoate) treatment resulted in high cumulative mortality and rapid mean time to death, leading to infected aphids not being detected by week 8 of the experiment. Under field conditions, the suspensions treatment showed PCM values of  $45.00 \pm 8.00\%$ ,  $34.21 \pm 15.37\%$ , and  $34.82 \pm 7.24\%$ , respectively. The MTD was  $6.00 \pm 2.00$ ,  $6.67 \pm 0.58$ , and  $7.42 \pm 0.58$  days, respectively, which was more efficient than the distilled water treatment. Pathogenic fungi have high potential for regulating aphid populations in both greenhouses and fields. The results of the experiment support the use of *P. neoaphidis* as a biocontrol agent to control aphids in commercial plant crops, despite insecticides quickly reducing the number of pests. Biocontrol methods are less toxic, more flexible than chemical pesticides, and have fewer negative impacts on human health and the environment. Biocontrol agents such as fungicides must be used preventatively.

## Acknowledgements

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## Conflicts of interest

The authors declare no conflict of interest.

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