International Journal of Agricultural Technology 2018 Vol. 14(3): 313-324
Available online http://www.ijat-aatsea.com
ISSN 2630-0192(Online)

Biodiversity and Investigating Pathogenic Levels of Endogenous Strains of *Pandora neaoaphidis* Collected from Cruciferous Crops in Northern Thailand

Kawpet, R.[1], De Bels, M.[2] and Saengyot, S.[3*]

[1]Faculty of Science and Technology, Suan Dusit University, Bangkok 10700, Thailand; [2]Program of Agriculture, Faculty of Science and Technology, Loei Rajabhat University, Loei 42000, Thailand; [3]Department of Plant Protection, Faculty of Agricultural Production, Mae Jo University, Chiang Mai 50290, Thailand.

Kawpet, R., De Bels, M. and Saengyot, S. (2018). Biodiversity and investigating pathogenic levels of endogenous strains of *Pandora neaoaphidis* collected from cruciferous crops in northern Thailand. International Journal of Agricultural Technology 14(3):313-324.

Abstract The biodiversity and virulence of aphid pathogen: *Pandora* (*Erynia*) *neoaphidis* from cruciferous crops covering thirteen districts in Chiang Mai, Lamphun and Lampang provinces, Northern of Thailand were evaluated. Base on phenotypic level, 128 isolates of *P. neoaphidis* were collected from various survey location. The result showed that natural occurrence pathogenicity rate of *P. neoaphidis* was in the range of 11 to 93.13% with an average of 41.22%±12.63. Three isolates (2.3%) showed pathogenic activity higher than 90% including Eyn13 was the most activity of 93.14±4.54 % followed by those of Eyn12 and Eyn14 which had the action of 91.68±5.13 % and 90.30%±23.57, respectively.In addition, these of Eyn04, Eyn17, Eyn16, Eyn20, Eyn15, Eyn24, Eyn02, Eyn18 and Eyn01 showed the natural pathogenic action in the range of 85.89±13.07 to 73.42±9.72, respectively. Contrary, pathogenicity rate of four isolates of *P. neoaphidis* (Eyn59, Eyn85, Eyn110 and Eyn62) were very low less than 15% which Eyn62 was the lowest (11.13%±5.94). As a whole, 39 isolates of *P. neoaphidis* established aphid pathogenic traits at 21 to 30% followed by those of 22 isolates at 31-40%, whereas the innate of 52 isolates had pathogenicity in the range of 40 to 100%, respectively.

Keywords: entopathogenic fungi, economic insect, *Pandora neaoaphidis*

Introduction

Aphids is important economic pests in various countries (Surendra and Semtner, 2006) which aphids' outbreak are causing of damaging of deformity and dropping of foliages, and yield reduction of 12.79 to 61.07% (El-Defrawi and El-Harty, 2010). In various countries, outbreak of cruciferous crops aphids such as *Brevicoryne brassicae* (L.), *Lipaphis erysimi*, *Myzus persicae*, root aphids, *Smynthurodes betae* and *Aphis gossypii* has been reported (Blackman and Eastop, 2000). However, in Thailand, several aphids e.g. cotton aphids (*A.*

[*] **Corresponding author:** Saengyot, S.; **Email:** sama_mju@yahoo.com

gossypii), cabbage aphids (*L. erysimi*), tobacco aphid (*M. persicae*) and corn aphids (*Rhopalosiphum maidis* (Fitch)) etc. had been found as reports of various studies (Rirkviree, 1967; Bänziger, 1976; Bänziger, 1977; Wongsiri, 1991). Moreover, aphids are transmitting vector of numerous viruses as well, for example, cotton aphids (*A. gossypii* Glover) causing viral disease in melon (Cucumis melo) (Sánchez *et al.*, 2001), cabbage aphids (*L. erysimi* (Kaltenbach) transmitting TuMV virus, and tobacco aphids (*Myzus persicae* (Sulzer)) carrying hyoscyamus virus III (henbane mosaic potyvirus - HMV) (Wang *et al.*, 1998) etc. In the present, several methods have been applied for aphids controlling including usage of pesticides. However, application of chemical substances causing of spreading of toxic substances and toxin to humans and environments both in short and long term periods. Some aphid species were insecticide-resistant strains such as tobacco aphids resisted organophosphate (Gould, 1966), lettuce aphid (Nasonovia ribis-nigri) tolerated acephate and methomyl substances in New Zealand (Moore and Hagerty, 2010; Barber *et al.*, 1999; Stufkens *et al.*, 2005) etc. depending on their genetics (Owain *et al.*, 2008). However, several natural mechanisms can regulate aphids population e.g. predators, parasitoids and fungal insect pathogens, for example, *Pandora neoaphidis*, *Entomophthora planchoniana* Cornu, *Beauveria bassiana* (Balsamo), *Conidiobolus obscurus* (Hall & Dunn) Remaudiere, *Neozygites fresenii* (Nowakowski) and *Aspergillus flavus* Link (Saengyot *et al.*, 2008; Chen *et al.*, 2008; Shah and Pell, 2003) etc. Nowadays, some of those fungal have been applied for aphids controlling as biological control agent in order to reduce in insecticide usages such as utilization of *Verticillium lecanii*, *B. bassiana* and *P.* (*Erynia*) *neophidis* (Copping, 2009) etc.

P. neoaphidis (Entomophthorales: Entomophthoraceae (Saengyot *et al.*, 2008), is an effective insect pathogen fungi which regulates various economic aphids, for instance, *A. gossypii*, *M. persicae*, and *L. erysimi* in several countries (Surendra and Semtner, 2006). According to report of Leskovar and Black (1994) showed that population of *A. gossypii* aphids were decreased after application of P. neoaphidis. This fungal population significantly associated with rising number of aphids (McLeod *et al.*, 1998). Generally, *P. neoaphidis* frequently occurs in almost any regions of the world (Chen *et al.*, 2008; Mcleod *et al.*, 1998). It grows considerably under warm weather and field conditions. Additionally, irrigations and application of insecticides had no effect on fungal proliferation as study of Leskovar and Black (1994). Although discovery in Thailand had been found that aphids were attacked by *P. neoaphidis*, no studies of biodiversity, distribution, special trait and virulences of this fungi to restrict target aphids under natural enviroments were encountered (Saengyot *et al.*, 2008).

Objectives: This study was to discover biodiversity of *P. neoaphidis* and collect infected aphids from cruciferous crops in northern of Thailand (Chiang Mai, Lamphun and Lampang provinces), and calculated percentage of destruction aphids under nature results in fungal biodiversity collections and useful information for further study and utilization of *P. neoaphidis* in Thailand.

Materials and methods

This research was carried out in Laboratory of Faculty of Science and Technology, Suan Dusit University, and Laboratory of Insect Diseases, Department of Plant Protection, Faculty of Agricultural Production, Maejo University, Thailand.

Collecting site and geography

The sample sites covered 3 provinces in Northern Thailand; including Chiang Mai Province (Mae Rim, Samoeng, San Sai, San Kamphaeng, Chom Thong and Saraphi districts), Lamphun Province (Mueang, Mae Tha and Ban Thi districts) and Lampang Province (Mueang Pan, Ngao, Mueang and Thoen districts) for discovering the variation of this fungi controlling aphid infested cruciferous crops. Chiang Mai is located between latitudes 17-21 ° N and longitudes 98-99 ° E. It is at 310 meters above sea level. Generally, the landscape is mostly flat plains near the Mae Ping River and is surrounded by mountains. The highest point in Chiang Mai is Doi Inthanon at 2,565.33 meters in Jomthong District. The weather is quite cool all year round with an average annual temperature of 25.4 ^{o}C. The annual rainfall in 2012 was 925.6mm. Lamphun Province is located at 18 ° N and 98-99 ° E, and is mainly surrounded with a flood plain; while there are mountainous plateaus in the northeast. Generally, the climate in Lamphun is not too hot or cold with temperatures ranging between 41 ^{o}C and 10 ^{o}C. The average annual rainfall is 925.6mm. Lampang Province stands above sea level at 268.80 m and is oval shaped consisting of several mountains and plateaus to its north and south. The average temperatures are between 41.7 ^{o}C and 9.7 ^{o}C with annual rainfall of 1168.1 mm in 2013. (Source: Bureau of Statistics. Office for National Statistics, 2013). (Figure 1)

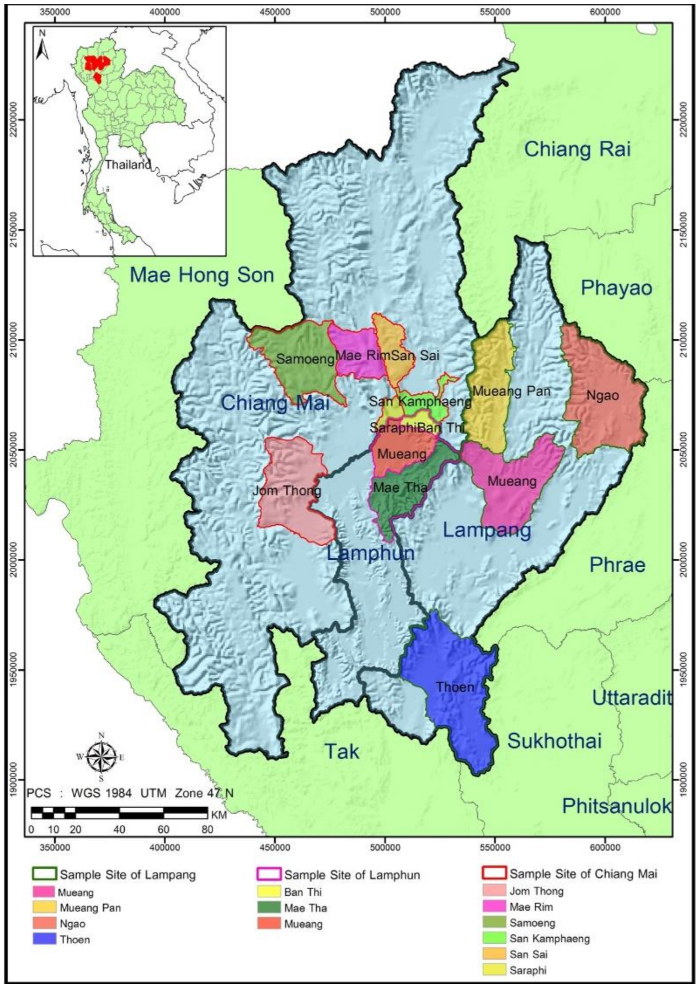

Figure 1. Map showing the sample site in the various provinces; Chiang Mai, Lamphun and Lampang

Estimating of natural occurrence pathogenic rate

P. neoaphidis isolations from aphid (residential insects) were collected. Number of non-infected and infected *P. neoaphidis* aphids were count based on phenotypic levels. Initial symptoms, aphids dead body was swollen, and became orange in comparison to non-infected yellow-green aphids. In each district, five locations were chosen (100 plants per site) by taking five leaves from bottom. Infection percentage of *P. Neoaphidis* infested aphids were calculated by equation below.

$$\text{Infection percentage of aphids} = \frac{\text{Number of infection aphids by } P.\ Neoaphidis}{\text{Number of existent aphids of each plant}} \times 100$$

Purification and isolation

Plants with dead aphids were cut into piece and kept in transparent plastic box of 6 X 3 cm width which holes of the top were sticked together with copper net to releasing moisture. Clean-sterilized tissue papers were put in bottom of container. Therefore, all samples were stored in cooler contained ices and took to laboratory. Fungal infected in plants with host aphids was isolated by dilution technique as standard method in PDA agar. Then, single appearance of fungal colony was collected according to its morphological colony: resemble white, or oranges-white colony with flat fibers in accordance with McLeod *et al*. (1998). The characteristics of fungal spores were investigated under microscopy at 10X (Saengyot *et al.*, 2008) and cultured on PDA agar for 5 days and incubated under room temperature (25 ± 2^{o}C). Fungal colony were recultured and each of remarkably single colony of P. neoaphidis was selected and kept as purify isolates in slant culture.

Results

Characteristics of infected aphids under field conditions

This survey found that aphids infected by Pandora neoaphidis displayed the following symptoms. Firstly, dead aphids were adhered to leaf blades due to the piercing of mycelium through the insect's body. Afterwards, the aphids' bodies were covered with fungal mycelium, then the dead bodies became orange because of the producing of abundant orange spores. Lastly, the pest's bodies dried and became dark orange. Spores of fungi spread to other aphids thus, leading to infection at this phase (Figure 2).

Survey of destruction assessment on aphids in nature

One hundred twenty-eight isolates of entomophotogenic fungi: *P. neoaphidis* were collected from sample sites. The result showed that 74 isolates (578%), 34 isolates (26.6%) and 20 isolates (15.6%) of *P. neoaphidis* were screened from Chiang Mai, Lamphun and Lampang provinces, respectively (Table 1). All fungal isolates were named as Eyn01 to Eyn128 (Table 1). It this study, pathogenicity rate of *P. neoaphidis* ranged from 11.32 to 93.13 percent with an average of 41.22 $\pm$ 12.63 (Figure 3). Furthermore, only three isolates (2.3%) of total isolates showed highly pathogenic activity destroying aphids in the range of 90-100%. In this sense, Eyn13 had the highest pathogenic action of 93.13±4.54% followed by Eyn12 and Eyn14 with 91.68±5.13 and 90.30±23.57%, respectively selecting from Maetha district, Lamphun province, which were higher than those of Eyn04 (85.89±13.07), Eyn17 (85.10±19.11), Eyn16 (84.89±13.07), Eyn20 (83.18±15.60), Eyn15 (83.02±14.40), Eyn24 (77.55±12.95), Eyn02 (75.89±26.11) Eyn18 (75.08±21.08) and Eyn01 (73.42±9.72), respectively.

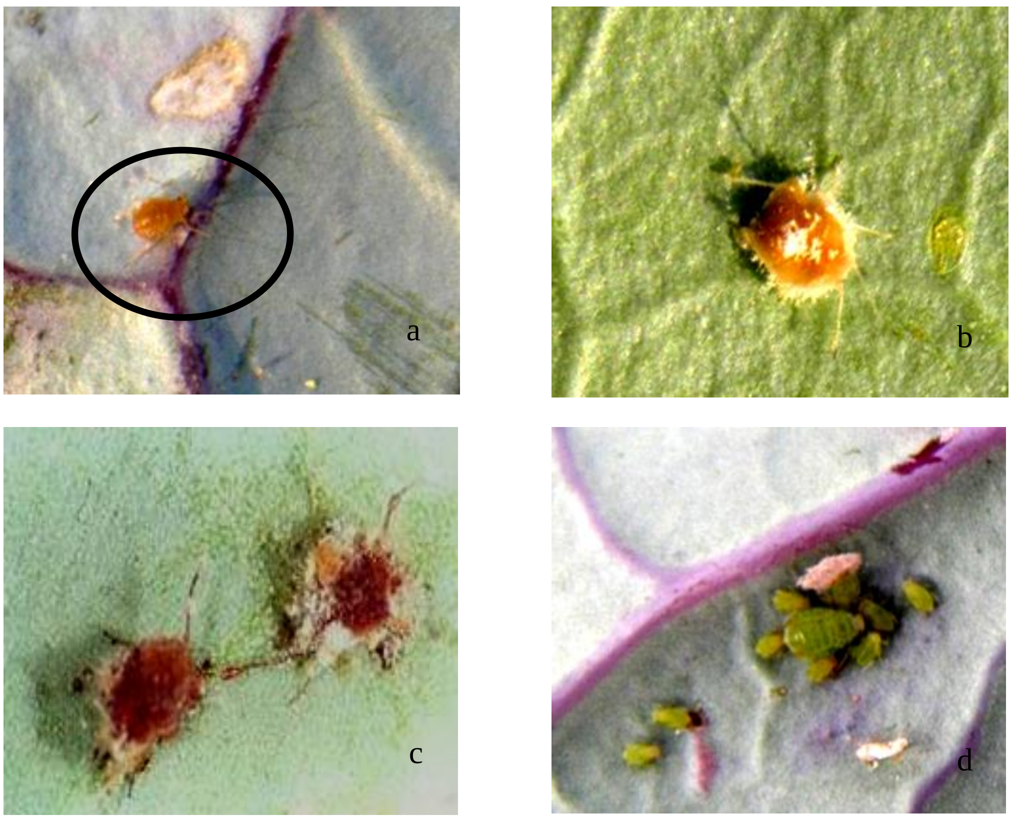

Figure 2. Characteristics of aphids which had been naturally destroyed by *P. neoaphidis* in a farmer's field including aphids that were destroyed in the first stage (a), the beginning of sporulation (b), finally, infected aphids were dried (c) in comparison to uninfected green aphids (d)

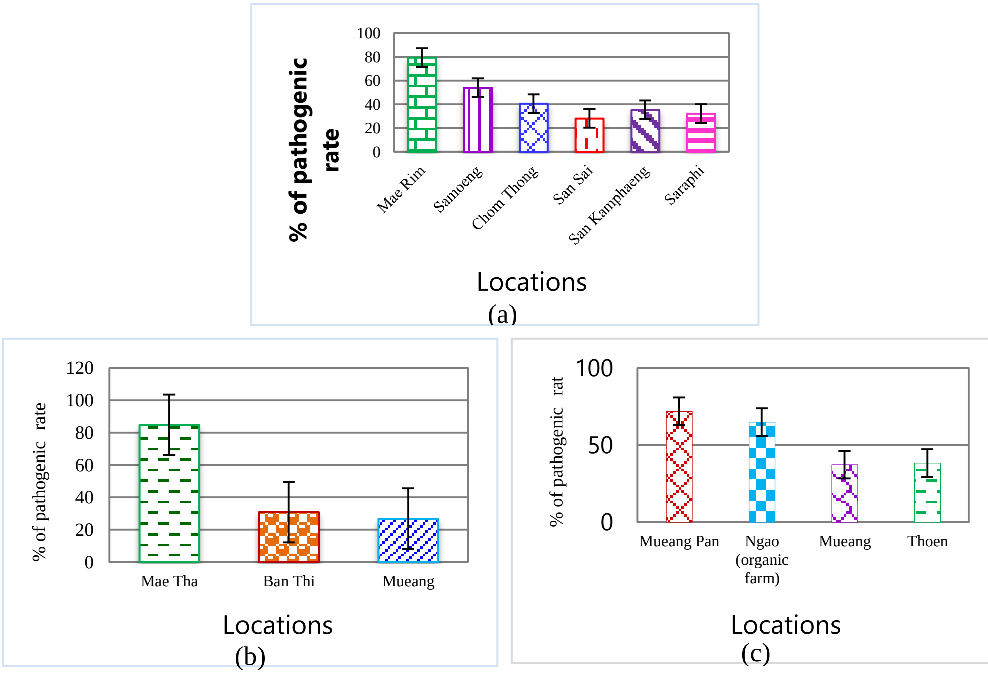

Figure 3. Natural occurrence pathogenic rate of *P. neoaphidis* against aphids surveying in cruciferous crop in Chiang Mai (a), Lamphun (b) and Lampang provinces (c)

Discussion

In this exploration, earlier results indicated that pathogenic activity and biodiversity of *P. neoaphidis* from cruciferous crops throughout Chiang Mai, Lamphun and Lampang provinces in northern region of Thailand varied depending on season, humidity, and topography. During low humidity and dry season were favoured condition for aphids outbreak, similar to the finding of Chen *et al.* (2008); Mcleod *et al.* (1998) that P. neoaphidis population frequency found in any regions in this world and all entire year around. Pathogenic activity of this fungus varied widely (11.13 to 9313%). Fungal population widely occurred both in highland (e.g. Khun Grang Royal Project, Doi Inthanon, Khong Hui Royal Project in Sameong dictrict, Mae Rim in Chiang Mai province, Mae tha in Lamphun, Mueang Pan, Ngao in Lampang) and lowland area (e.g. Ban Thi, Mueang Lamphun sample sites). It seem to showed that 47.66% (61 isolates) of *P.neoaphidis* isolates had pathogenic action in the range of 21 to 40% which most of them were collected from lowland regions like San Sai, San Kamphaeng, Saraphi in Chiang Mai and

Mueang Lamphun provinces. The earlier result indicated that Eyn12, Eyn13 and Eyn143 collected from Maetha district, Lamphun province had highly pathogenic rate (91.68±5.13 to 93.13±4.54%). Moreover, 28.13% of P. neoaphidis (36 isolates) had pathogenic activity above 50%, particularly most of isolations of Mae Tha (Eyn11 to Eyn15), Mae Rim (Eyn16 to Eyn20), Samoeng (Eyn23 to Eyn25), Mueang Pan (Eyn01 to Eyn05), Ngao districts (Eyn06 to Eyn10), respectively. For the reason, it may be due to equilibrium of natural environments (various type of natural enemy mechanisms: predators, parasitoids and fungal insect pathogens (Chen *et al.*, 2008; Shah and Pell, 2003), high humidity and optimum weather were more desirable for promoting fungal growth and aphids controlling than those at other location isolates (Table 1 and 2). While, Eyn62, Eyn85 and Eyn110 had the lowest pathogenicity rate (11.32±11.03) owing to frequency cultivation of various crops in San Sai district. Additionally, the survey tend to show that variation of activities of *P. neoaphidis* infested aphids among various locations were found as well. For instance, Eyn11 to Eyn15 from Mae Tha, Lamphun had high ability ranging from 66.20±15.06 to 93.13±4.54, while Eyn110 to Eyn 114 from Sansai district, Chiang Mai gave aphids destruction activity in the range of 11.32±11.03 to 29.66±10.63% etc. Additionally, 14 isolates from Chom Thong had widely restraining ability of 18.46 to 71.52% (Table 1) which were higher than any other areas selecting in Chiang Mai.

However, in some areas, the ability of natural pathogenic fungi did not considerably differ than those of other locations e.g. Eyn16 to Eyn19 from Mae Rim district (68.89±13.07 to 85.10±19.11) and Eyn36 to Eyn40 from Mueang Lamphun (23.98 to 31.78%) etc. Furthermore, *P. neoaphidis* either screened from same district or sample site had different pathogenic activity rates on aphids. In case of isolate from Chom Thong no. 1, Eyn74 gave capability of 19.57±10.25, whereas Eyn95 had activity of 71.52±11.11%. At San Kamphaeng sample site no. 4, activity of Eyn102 was low at 17.78±11.41%, while activity of Eyn120 was high at 71.52±11.11% etc. This finding was similar to report of Sierotzki *et al.* (2000). They found that physiological mechanisms of *P. Neoaphidis* e.g. insect host range, infection levels, germination rates and favorable temperature were difference among species or isolates. In particular, this exploration obviously indicated that destruction capability of entomopathogenic fungi: *P. neaoaphidi* screening from cruciferous crops covering three provinces rather differed depending on genetic variation under various habitats as well, similar to report of Hoskin *et al.* (2005). Variation of genetic, infection host insects, fungal diseases virulence in nature of entomopathogenic fungi resulted from duration and evolution in specification geography (Owain *et al.*, 2008), e.g. geographic isolation, geographic barriers genetic drift, mutation and gene pool of fungi. These greatly influenced increasing of specific - fungal evolution, various genetics biodiversity as well as microbial genetics. In addition, these characters induced highly survival adaptation under changeable stressful environments by creating special structures such as thick-walled spores, cysts and capsular formations, which tolerate the drought, heat, chemical substance, radiation and adapted compulsion in different habitats, growth rate and its virulence (Nielsen *et al.*, 2007). Cupriferous crops are economic vegetables, and are often cultivated year round in northern Thailand.

Therefore, appropriate conditions of *P. neoaphidis* distribution in various plantations is needed. For further study, researchers should investigate an optimized medium for purification, rate and growth rate of a single colony; the quantity of sporulation and subsistent spores in difference mediums, which are closely associated with fungal virulence; and the capability of *P. neoaphidis* to restrict the aphid population as a biological control as in the report of Papierok and Hajek (1997). Furthermore, the study of increasing mass culture production and potentiality of this fungus in the laboratory in order to gain useful information are necessary. The advantage of biodiversity of high effective and wide adaptation of entomopathogenic fungus: *P. neoaphidis* under various environments is an alternative noteworthy procedure as microbial pesticides on controlling various aphid species for cruciferous crop in term of biological control which is safety for agricultural system, environmentally friendly as well, which is a sustainable approach of aphids controlling for Thailand.

Acknowledgement

This work was financially supported by Suan Dusit University, under National Research Council of Thailand (NRCT).

References

Bänziger, H. (1976). Keys for the identification of aphids (Homoptera) I, Winged aphids of species economically important in Thailand. Department of Agriculture and UNDP/FAO. Plant Protection Service Technical Bulletin No 36, Bangkok.

Bänziger, H. (1977). Keys for the identification of aphids (Homoptera) II. II. *Field identification* No. 37. Department of Agriculture and UNDP/FAO, Bangkok.

Barber, M. D., Moores, G. D., Tatchell, G. M., Vice, W. E. and Denholm, I. (1999). Insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae) in the UK. Bulletin of Entomological Research 89:17-23.

Blackman, R. L. and Eastop, V. F. (2000). Aphids on the world' crops: An identification and information guide. New York: John Wiley & Sons.

Chen, B., Li, Z. Y. and Feng, M. G. (2008). Occurrence of entomopathogenic fungi in migratory alate aphids in Yunnan Province of China Province of China. Biocontrol 53:317-326. doi: 10.1007/s10526-006-9063-z.

Copping, L. G. (2009). The manual of biocontrol agents (4th ed.). Hampshire, British Crop Protection Council, UK.

El-Defrawi, G. M. M. and El-Harty, E. H. (2010). Injury levels and yield loss model for the cowpea aphid *Aphis craccivora* Koch on *Vicia faba* (L). Available source: http://faculty.ksu.edu.sa/ehab/Pictures.

Gould, H. J. (1966). Organophosphorus insecticide resistance in aphids on year-round chrysantimums. Plant Pathology 15:109-112. DOI: 10.1111/j.1365-3059.1966. tb00325.x

Hoskin, C. J., Megan, H., McDonald, K. R. and Craig, M. (2005). Reinforcement drive rapid allopatic. Nature, 437 (7063), 1353–1356. Available source: http://www.aphidwatch. com/ lettuce/aphid-resis2.pdf, June 6, 2016. doi:10.1038/nature04004.

Leskovar, D. I. and Black, M. C. (1994). White rust infection leaf chlorosis in relation to crop strategies in spinach. Journal Environmental and Experimental Botany 34:363-370. doi:10.1016/0098-8472(94)90018-3.

McLeod, P. J., Steinkraus, D. C., Correll, J. C. and Morelock, T. E. (1998). Prevalence of *Erynia neoaphidis* (Entomophthorales: Entomopthoraceae) infection of green peach aphid (Homoptera: Aphididae) on spinach in the Arkansas River Valley. Environmental Entomology 3:796-800.

Moore, M. S., Bayer (NZ) Ltd. and Hagerty G. C. (2010). Preliminary advice on minimizing insecticide resistance in lettuce aphids. Available source: http://www.aphidwatch.com /lettuce/aphid-resis2.pdf, July 5, 2016.

Nielsen, C., Jensen, A. B. and Eilenberg, J. (2007). Survival of entomophthoralean fungi infecting aphids and higher flies during unfavourable conditions and implications for conservation biological control. In: Ekesi, S. and Maniania, NK. (Eds), Use of Entomopathogenic Fungi in Biological Pest Management. Research Signpost Trivandrum, India.

Owain, R. E., Franzmann, B., Thackray, D. and Micic, S. (2008). Insecticide resistance and implications for future aphid management in Australian grains and pastures: a review. Journal of Experimental Agriculture 48(12): 1523–1530. Available source: http://dx.doi.org/10.1071/EA07426, May 8, 2015.

Rirkviree, S. (1967). Some aphids of economic importance in Thailand. (Bachelor’s thesis). Kasetsart University.

Saengyot, S., Channarong, D., Woravan, C., Warunee, S. and Porchai, D. (2008). Survey, collecting and evaluating of microbial for insect control and weed control. National Biological Control Research Center, Bangkok.

Sánchez, M. V., Agüero, R. and Rivera, C. (2001). Host plants of *Aphis gossypii* (Aphididae), vector of virus of *Cucumis melo* melon (Cucurbitaceae) in Costa Rica. Revista de Biología Tropical 1:305-11.

Shah, P. A. and Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 61:413-423. DOI 10.1007/s00253-003-1240-8.

Sierotzki, H., Camastral, F., Shah, P., Aebi, M. and Tuor, U. (2000). Biological characteristics of selected *Erynia neoaphidis* isolates. Mycological research 2:213-219.

Stufkens, M. A. W., Walker, G. P. and Martin, N. A. (2005). Lettuce aphid resistance management and prevention Strategy. In N.A. Martin, Beresford Palmerston North

Pesticide Resistance: Prevention and Management Strategies 2005 Hastings. New Zealand Plant Protection Society Inc, New Zealand.

Surendra K. D. and Semtner, P. J. (2006). Within-Plant Distribution of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) in Populations of the Tobacco-Feeding Form of *Myzus persicae* (Homoptera: Aphididae) on flue-cured Tobacco. Journal of Agricultural and Urban Entomology 2:65-76.

Wang, R. Y., Powell, G., Hardie, J. and Pirone, T. P. (1998). Role of the helper component in vector-specific transmission of potyviruses. Journal of Clinical Virology 79:1519-1524.

Wongsiri, N. (1991). List of insect, mite and other zoological pests of economic plants in Thailand. Technical Bulletin. Entomology and Zoology Division, Department of Agriculture, Bangkok.

(Received: 5 March 2018, accepted: 30 April 2018)