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1 Pyrethroid insecticides susceptibility of *Stomoxys calcitrans* (Linnaeus) and *Stomoxys*
2 *indicus* Picard (Diptera: Muscidae) from cattle farms in southern Thailand

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23 **Abstract**

24

25 The susceptibility to six pyrethroid insecticides (permethrin, deltamethrin, alpha-
26 cypermethrin, cypermethrin, lambda-cyhalothrin and bifenthrin), each at the recommended
27 concentration, was evaluated for the two stable fly species *Stomoxys calcitrans* (Linnaeus)
28 and *Stomoxys indicus* Picard, through tarsal contact using a World Health Organization
29 (WHO) cone bioassay procedure. The field populations of stable flies were collected from
30 three study sites (Songkhla, Phattalung and Satun provinces) in Thailand. The stable flies
31 were exposed to insecticide-treated filter paper for 30 min and their knockdown counts at 30
32 min and 60 min and mortality counts at 12 hr and 24 hr were recorded. The *S. calcitrans* and
33 *S. indicus* in Songkhla and Phattalung populations were moderately susceptible to pyrethroids
34 for 24-hr mortality. Nonetheless, the Satun population of *S. indicus* was completely
35 susceptible to permethrin with 100% mortality and the lowest susceptible to deltamethrin and
36 bifenthrin. The results indicate a generally low susceptibility of stable flies to pyrethroids in
37 the southern provinces of Thailand.

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39 **Keywords:** insecticide susceptibility, pyrethroid, stable fly, *Stomoxys calcitrans*, *Stomoxys*
40 *indicus*

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42 **Introduction**

43 Stable flies (Diptera: Muscidae) are known as common blood-sucking insect pests
44 worldwide. They are among the most important globally recognized pests that impact
45 livestock and wildlife health. There are two common species for diurnal and crepuscular
46 activity abundance in Thailand namely, the cosmopolitan species *Stomoxys calcitrans*
47 (Linnaeus 1758) and *Stomoxys indicus* Picard, respectively (Foil and Hogsette 1994;
48 Tainchum et al. 2010, Changbunjong et al. 2012, Keawrayup et al. 2012, Lorn et al. 2020).
49 Stable flies inflict painful bites that annoy, stress and interrupt feeding to livestock, causing
50 loss of blood, lactation and weight loss (Campbell et al. 1987). In 1994, Foil and Hogsette
51 (1994) stated that a 28% weight loss was recorded in livestock attributed to the direct feeding
52 and various defensive behaviors against the stable fly bites all day long. These insects are
53 biological vectors and mechanical carriers of particular pathogens, which cause problems in
54 animal production systems (Changbunjong et al. 2012, Baldacchino et al. 2013). Moreover,
55 stable fly bites can be a considerable annoyance to humans, especially along the beaches in
56 West Florida where they can severely affect the tourist industry. In the USA, economic
57 losses caused by stable flies were estimated at US\$432 million in 1991 (Kunz et al. 1991) and
58 the cost for stable fly control was estimated at over US\$100 million (US\$9.80 per head) in
59 1993. The estimated economic losses in the United States, caused by stable flies, were US\$1
60 billion per year (Taylor and Berkebile 2006, Taylor and Berkebile 2008), and the reduction of
61 animal productivity cost over US\$2.2 billion in 2012 (Taylor et al. 2012).

62 Stable flies are controlled by many methods, but the main method relies on
63 insecticides. However, several studies have reported that insecticides did not provide
64 effective stable fly control (Marcon et al. 1997, Pitzer et al. 2010, Reissert-Oppermann et al.
65 2019). The reduced susceptibility to insecticides in a fly population is explained by a
66 selection effect that leads to resistant fly strain (Cilek and Greene 1994). In 1958, insecticide

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resistance of stable fly to DDT, dieldrin, dilan and methoxychlor has been recorded (Brown et al. 1971). The resistance in a stable fly to pyrethroid (permethrin) and organophosphate (dichlorvos and stirofos) was first reported in Kansas (Cilek and Greene 1994). A few years later, stable flies from southeastern Nebraska, USA, showed low susceptibility to permethrin, stirofos, methoxychlor (Marcon et al. 1997) and a population from Florida was also reported to have low susceptibility to permethrin (Pitzer et al. 2010). Then *S. calcitrans* isolated in southwestern France was resistant to five pyrethroids (cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin and permethrin) (Salem et al. 2012). Recently, Olafson et al. (2019) reported that stable fly from Nakhon Ratchasima province, Thailand, was resistant against pyrethroid.

The pyrethroid insecticides are typically highly toxic to insect pests but harmless to mammals. Over the past decade, pyrethroids have been used in Thailand to control insect pests in both the agricultural and public health sectors. There is a series of registered products, from pyrethrin to synthetic pyrethroid insecticides, launched for the public as well as residential uses that may induce urban runoff and water contamination, as well as deposits in the soil. However, there is no prior report on the susceptibility of stable flies in Thailand to insecticides, or on the level of resistance in stable fly populations. The current study addresses these gaps in knowledge, especially regarding pyrethroid insecticides that are widely used in and around agricultural farms and by households.

Materials and Methods

Stable fly sampling

Adults of two common local species of stable flies in southern Thailand (Lorn et al. 2020), namely *Stomoxys calcitrans* and *Stomoxys indicus*, were collected from three localities in Southern Thailand (Songkhla: SON, Phattalung: PHA and Satun: SAT provinces) during

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September-November 2018 (Table 1; Fig. 1). These three study sites have been known for organic cattle farm learning centers, and on two farms that belong to universities insecticide treatments for pest control are applied, while one farm pursues organic farming practices. Adults of both sexes of the stable flies were captured between 0800 and 1800 hours, with a sweeping net and a mouth aspirator and they were kept alive and transferred to clean insect cups and later to insect cages. Morphological identification of species was individually performed under a stereomicroscope, following the taxonomic keys (Changbunjong et al. 2012, Baldacchino et al. 2013) for sorting by species to *S. calcitrans* and *S. indicus*. The collected alive specimens were kept in net cages and were provided ad libitum as nutrition — 100% organic honey solution and water-soaked on a cotton ball. The cages were placed in an insectary at 27°C and 80% RH in the Pest Management Laboratory, Faculty of Natural Resources, Prince of Songkla University, Hat Yai Campus, Songkhla Province, Thailand, for a couple of days for their blood meal digestion before bioassay test.

Insecticide-treated paper preparation

Insecticide-treated filter papers were prepared by using acetone solutions of synthetic pyrethroid insecticides, including a technical grade of permethrin (92.15% purity), deltamethrin (99.16% purity), alpha-cypermethrin (97.34% purity), cypermethrin (93.52% purity), lambda-cyhalothrin (97.16% purity), bifenthrin (98.35% purity) (Sherwood Chemicals Public Company Limited, Suan Luang, Bangkok) and commercial-grade cypermethrin (25.00% w/v) (Intergrade Trading Co. Ltd, Bangkapi, Bangkok). Absolute acetone (99.99%) was used as the solvent to dissolve each insecticide, and as the negative control. Rectangular sheets of filter paper measuring 10 x 10 cm (Whatman® No. 1) were impregnated with 1 mL of each solution, based on the recommended concentration of each technical grade insecticide: permethrin (0.5500% w/v), deltamethrin (0.0500% w/v), alpha-

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cypermethrin (0.0750% w/v), cypermethrin (0.2500% w/v), lambda-cyhalothrin (0.0750% w/v) and bifenthrin (0.0625% w/v), and one commercial-grade insecticide cypermethrin (25.00% w/v) for residual treatment of resting-site for fly control (ODOC5 2018). The treated papers were stored at 4 °C and each was used only once.

WHO cone bioassay for residual contact test

The tests were performed with slight modifications according to Tainchum et al., (2018). Insecticide susceptibility bioassays were performed using World Health Organization (WHO) cone test kits (WHO 2006). The WHO cone was placed on a filter paper for actual treatment or control. Five stable flies (both sexes) of each species were exposed to an insecticide-treated paper for 30 min and then transferred to clean plastic cups (5 individuals per cup), and provided with a cotton pad soaked with water and honey on the lid of the cup. Six replicates were carried out for each insecticide susceptibility test. The numbers of knocked-down (KD) flies were recorded, for a rapid knockdown at 30 min and a regular one at 60 min, and mortality was observed at 12 and 24 hr after treatment.

Data analysis

The insecticide susceptibility status of stable flies was evaluated and interpreted following the revised WHO criteria (WHO 2016). The observed mortality was adjusted by Abbott's formula when the control mortality was greater than 5% and less than 20% (Abbott 1925). The interpretation of percent mortality of tested stable flies at 24hr after insecticide exposure was labeled as one of susceptible (mortality 98-100%), incipient resistance (mortality 90-97%), and resistant (mortality below 90%) (WHO 2016). The responses of stable flies were compared between knockdown times (30 min and 60 min) and mortalities (12 hr and 24 hr) by calculating the differential knockdown time (DKT) = percent

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knockdown at 60 min - percent knockdown at 30 min and the differential mortality time (DMT) = percent mortality at 24hr - percent mortality at 12hr. A positive differential response indicates no recovery of the tested stable flies, while a negative value indicates recovery from knockdown or apparent mortality.

The mean values of percent knockdown and mortality were tested for normality of which the Shapiro-Wilk test ($P>0.05$) and were compared using an ANOVA at 5% of probability. The means were separated using Tukey's significant difference test if the ANOVA statistic was significant ($P<0.05$). The analysis was performed using the SPSS software for Windows (version 10; SPSS Inc.; Chicago, IL, USA).

Results

Pyrethroid susceptibility of two stable flies (*S. calcitrans* and *S. indicus*) was assessed by the WHO cone bioassay. This is an initial report on monitoring the distribution of pyrethroid insecticide effectiveness against wild stable fly populations in southern Thailand. The results of bioassays are shown in Tables 2-3. Overall, the knockdown at 60 min after exposure was in the range 73.33-100% across all tested populations of *S. calcitrans* and all insecticides, whereas *S. indicus* had 40-100% knockdown levels. The mortality range was from 26.67% to 95.83% and from 13.33% to 100% for *S. calcitrans* and *S. indicus*, respectively.

The *S. calcitrans* SON population, exhibited a high level of knockdown by all the insecticides (>90%) except for bifenthrin that gave 80.83% knockdown. A complete knockdown was found in samples tested with deltamethrin and lambda-cyhalothrin. The highest mortality rate at 24 hr of tested *S. calcitrans* populations to a pyrethroid was found in the SON population with bifenthrin at 95.83%, while the mortality caused by other insecticides was below 90% (Table 2). Commercial cypermethrin provided complete

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knockdown (100%) of *S. calcitrans* in the PHA population. The high knockdown level (>90%) of *S. calcitrans* in the PHA population was found from exposure to deltamethrin, alpha-cypermethrin, and lambda-cyhalothrin. Similar to the SON strain, the highest mortality in samples of *S. calcitrans* was caused by bifenthrin (86.67% mortality) (Table 2).

For *S. indicus*, the knockdown observed at 60 min after exposure to insecticide was the highest in both PHA and SAT populations with permethrin. The SAT population when tested with alpha-cypermethrin had a high 96.67% knockdown rate, while relatively low knockdown levels were found with lambda-cyhalothrin in both PHA and SAT populations. The highest mortalities of both *S. indicus* populations were found when exposed to permethrin, namely 96.67% and 100% for samples collected from PHA and SAT, respectively. These were considered to have incipient resistance in the PHA strain while the SAT strain was resistant. There was no significant difference in mortality rates of PHA strain when tested with permethrin and bifenthrin, but they gave higher mortalities than the other insecticides (Table 3). Mortalities caused by all other insecticides in the SAT population, except for permethrin, were not significantly different. From these results, it is obvious that all tested stable flies (strains from all sampled locations) were resistant to all pyrethroid insecticides, except for *S. indicus* from both PHA and SAT when exposed to permethrin (Table 2–3).

The differential mortality responses of *S. calcitrans* by time (24hr and 12hr) was the largest in samples collected from PHA when exposed to bifenthrin (51.72%), while the differential in knockdown by time (60 min and 30 min) was the largest (82.14%) in samples collected from SON tested with bifenthrin (Fig. 2A–B). In *S. indicus*, the largest differential mortality found was in the PHA population exposed to bifenthrin (13.33%). Similar to *S. calcitrans*, the differential knockdown by time was also the highest in samples of *S. indicus* from PHA with bifenthrin (63.33%), followed by lambda-cyhalothrin (-34.48%) (Fig. 2C–D).

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Comparability between the pyrethroid insecticide response of two species, four populations of stable fly

The pyrethroid insecticide response of four populations was performed by Turkey's multiple range test ($P < 0.05$) (Table 4). All four populations, devoid of species and province, were compared for the mortality rate (%). There was no significant difference in the mortality rate of stable flies exposed to alphacypermethrin, cypermethrin (Tec), cypermethrin (Com), and negative control. Completely susceptible to permethrin, 100% mortality was seen in *S. indicus* SAT which was significantly different with another 3 populations (73.33% mortality). While *S. indicus* SAT showed the significantly lowest mortality rates in the deltamethrin and bifenthrin were less than half of the *S. calcitrans* SON, *S. calcitrans* PHA and *S. indicus* PHA.

Discussion

Inappropriate and excessive use of insecticides causes contamination in the environment and induces resistance in arthropods. Their metabolites result in a reduction of contamination events and increase the challenge for those who are involved in livestock production, along with insecticide resistance issues (Brito et al. 2018). Adult stable flies can be exposed to insecticides that are used during outbreak situations, such as insecticidal fogging and residual spraying to reduce the impacts of stable flies on animals. Exposure can also occur through programs for controlling other livestock ectoparasites, such as house flies, *Musca domestica* Linnaeus (Olafson et al. 2019). The resistance of stable flies to commonly used insecticide classes has been previously reported for organochlorines, organophosphates, and pyrethroids in the U.S. and some European countries (Somme 1958, Cilek and Greene 1994, Pitzer et al. 2010). Synthetic pyrethroids have been used extensively in Thailand for the control of agricultural and livestock pests, and also against disease vectors (Pakvilai et al. 2012, Chareonviriyaphap et al. 2013, Wongta et al., 2018). Resistance to pyrethroids was

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first reported in stable flies in Kansas cattle feedlots in 1994 (Cilek and Greene 1994) and the presence of resistant stable fly populations in another area was reported later on (Pitzer et al. 2010, Salem et al. 2012, Tainchum et al. 2018). No prior study of the susceptibility of stable flies to insecticides has been done in Thailand. No standard bioassay has yet been recommended for stable fly testing, unlike for the testing of mosquitos or house flies. In addition, this is the first report to study on insecticide susceptibility status of *S. indicus*. This fly species was found to be the second-most numerous species in all the study sites, and showed crepuscular activity in a previous report (Lorn et al. 2020); therefore, a study on susceptibility to insecticides of this species was worthwhile.

This study was carried out to investigate the status of pyrethroid susceptibility in stable flies sampled from different sites in the southern part of Thailand. Our findings show that *S. calcitrans* was the most susceptible to bifenthrin, while *S. indicus* was the most susceptible to permethrin. Bifenthrin is a non-alpha-cyano pyrethroid insecticide and an acaricide recommended by the WHO for indoor residual spraying. It has a relatively low irritant and knockdown effect on mosquitoes; thus allowing the mosquitoes to rest on treated surfaces for a longer period for exposure to a lethal dose, and hence provides a high mortality rate (Komalamisra et al. 2009). It is a newly synthesized pyrethroid and its effectiveness has been studied preliminarily in Thailand, in 1998 (OVBDC 1999). These properties could have an impact on the stable fly population density if complete spraying coverage is achieved in a community. The most permethrin susceptibility in this study was seen in *S. indicus*. Where a new combination of fipronil and permethrin provides excellent repellency and insecticidal efficacy for at least 5 weeks against *S. calcitrans* (Fankhauser et al. 2015). The knockdown resistance (*kdr*) associated with permethrin resistance was detected in stable flies from the United States, Costa Rica, France and Thailand (Olafson et al. 2019). Regarding the susceptibility to pyrethroids, the results from this current study demonstrate that the

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recommended concentration of any of the phenotypical tested pyrethroids (recommended concentration of commercial-grade) was not completely able to control the two field populations of *S. calcitrans*, while interestingly *S. indicus* in the Satun sample was still susceptible to permethrin. The collection site in Satun Province was located in an organic local cow farm, not treated with an insecticide. However, resistance to most tested pyrethroids was occurring in samples collected from this site. This may be explained by the dispersal of resistant stable flies that may happen more readily on nearby dairy farms or agricultural areas, where such selection pressure was present, impacting the effectiveness of chemical control at nearby dairies. The low susceptibility of stable flies to pyrethroids seen in this study suggests that current control practices relying on these insecticides are inadequate for further deployment. The recommended concentrations of pyrethroids for controlling stable flies should be adjusted to achieve proper control and elimination. However, the susceptibility of stable flies in other areas of Thailand has not been reported. The susceptibility of different populations of stable flies to insecticides may be affected by various factors, such as geographic variations, breeding habitat, health and age of stable flies, and pattern of insecticide use. Further investigations are needed to identify the susceptibility and the potential forces driving resistance in stable flies in Thailand.

The insecticide resistance of stable fly in this study may have consequences to the emerging of cattle and buffalo diseases along with southern Thailand, especially arthropod-borne viral (arboviruses) diseases -lumpy skin disease (LSD), meaning that the ordinary insecticide control program is not as effective as it could be. The first case occurred on 29 March 2021, an LSD outbreak involving beef cattle farms in Roi-Et Province, Northeastern Thailand (Arjkumpa et al. 2021). Then the outbreak was spread all around the country. The FAO team reported that the economic impact of LSD on South, East and Southeast countries was estimated to be up to USD 1.45 billion in direct losses of livestock and production.

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Vaccination could assist to prevent this LSD but still an absence of large-scale vaccination targeting susceptible livestock (Roche et al. 2020). So the effective vector control can be a support to improve the reduction of disease transmission between host-vector contact.

Insecticide resistance develops with an evolutionary process of natural selection, which adapts that insect population to insecticides. The main mechanisms involve either mutation within the target site of the insecticide and/or an increased rate of insecticide detoxification (Nkya et al. 2013). To confirm insecticide resistance in a stable fly population, molecular techniques could assist in identifying the prevalence of such modifications in each population of stable flies. The recent report by Olafson et al. (2019) indicated that a low frequency of the *kdr* allele was observed in a stable fly population from the Wang Nam Khiao, Nakhon Ratchasima, Thailand. The resistance mechanisms should be elucidated to support estimating resistance and to design an effective strategy for the proper control of the target pests. Moreover, standard insecticide resistance detection assays for stable flies should be developed, with improved protocols, to facilitate collecting baseline information.

Further classes of insecticides should be examined and monitored for generating complementary data on the level of resistance, and possible evolution in the level of resistance in stable flies should be observed. The control of stable fly populations could be achieved by using an integrated approach or integrated vector management (IVM), with innovations in trapping, sanitization measures, parasitoid insects, botanical insecticides, and the application of efficient chemical compounds.

Conclusion

The phenotypic pyrethroid insecticide susceptibility conclusively demonstrates mostly resistance in stable fly populations of the southern provinces in Thailand. This study indicates that the stable fly populations in Thailand should be further monitored, not only regarding the

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current insecticide used to protect livestock but also for new semiochemical toxicity and/or for different mechanisms of insecticide resistance, to improve the effectiveness of stable fly control. Also, new standard techniques for insecticide susceptibility detection in the stable fly samples should be considered, as these could be essential tools to consistently measure insecticide resistance.

Conflict of interest

The authors declare no conflict of interest in connection with this work.

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References

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J.Econ. Entomol. 18: 265 – 267.
- Arjkumpa O., M. Suwannaboon, M. Boonrawd, I. Punyawan, P. Laobannu, S. Yantaphan, A. Bungwai, V. Ponyium, N. Suwankitwat, P. Boonpornprasert, B. Nuansrichay, S. Kaewkalong, D. Ounpomma, W. Charoenlarp, N. Pamaranon, R. Prakottheo, N.

Lorn et al. 2021- Pyrethroid susceptibility of *Stomoxys* spp.

- 313 Buameetoop, V. Punyapornwithaya, and T. Songkasupa. 2021. First emergence of
- 314 lumpy skin disease in cattle in Thailand, 2021. *Transbound Emerg. Dis.* 68: 3002-
- 315 3004.
- 316 Baldacchino, F., V. Muenworn, M. Desquesnes, F. Desoli, T. Charoenviriyaphap, and G.
- 317 Duvallet. 2013. Transmission of pathogens by *Stomoxys* flies (Diptera, Muscidae): a
- 318 review. *Parasite.* 20: 26.
- 319 Brito, L. G., F. S. Barbieri, R. B. Rocha, A. P. L. Santos, R. R. Silva, E. S. Ribeiro, F.
- 320 Guerrero, L. Foil, and M. C. S. Oliveira. 2019. Pyrethroid and organophosphate
- 321 pesticide resistance in field populations of horn fly in Brazil. *Med. Vet. Entomol.* 33:
- 322 121–130.
- 323 Brown, A. W. A., R. Pal, and World Health Organization. 1971. Insecticide Resistance in
- 324 Arthropods 2nd ed. World Health Organization, Geneva.
- 325 Campbell, J. B., I. L. Berry, D. J. Boxler, R.L. Davis, and D. C. Clanton. 1987. Effects of
- 326 stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. *J.*
- 327 *Econ. Entomol.* 80: 117–119.
- 328 Changbunjong, T., T. Weluwanarak, P. Ratanakorn, P. Maneeon, M. Ganpanakngan, C.
- 329 Apiwathnasorn, S. Sungvornyothin, P. Sriwichai, S. Sumruayphol, and J.
- 330 Ruangsittichai. 2012. Distribution and abundance of Stomoxyini flies (Diptera:
- 331 Muscidae) in Thailand. *Southeast Asian J. Trop. Med. Public Health* 43: 1400–1410.
- 332 Chareonviriyaphap, T., M. J. Bangs, W. Suwonkerd, M. Kongmee, V. Corbel, and R. Ngoen-
- 333 Klan. 2013. Review of insecticide resistance and behavioral avoidance of vectors of
- 334 human diseases in Thailand. *Parasit.Vectors.* 6: 280.
- 335 Cilek, J. E., and G. L. Greene. 1994. Stable fly (Diptera: Muscidae) insecticide resistance in
- 336 Kansas cattle feedlots. *J. Econ. Entom.* 87: 275–279.

Lorn et al. 2021- Pyrethroid susceptibility of *Stomoxys* spp.

- 337 Fankhauser, B., J. P. Irwin, M. L. Stone, S. T. Chester and M. D.. Soll. 2015. Repellent and
338 insecticidal efficacy of a new combination of fipronil and permethrin against stable
339 flies (*Stomoxys calcitrans*). Parasit.Vectors. 8: 61.
- 340 Foil, L. D. , and J. A..Hogsette. 1994. Biology and control of tabanids, stable flies and horn
341 flies. Rev. Sci. Tech. 13: 1125–1158.
- 342 Keawrayup, S., G. Duvallet, S. Sukonthabhirom, and T. Chareonviriyaphap. 2012. Diversity
343 of *Stomoxys* spp. (Diptera: Muscidae) and diurnal variations of activity of *Stomoxys*
344 *indicus* and *S. calcitrans* in a farm, in Wang Nam Khiao District, Nakhon Ratchasima
345 Province, Thailand. Parasite. 19: 259–265.
- 346 Komalamisra, N., R. Srisawat, C. Apiwathanasorn, Y. Samung, and P. Kaisri. 2009. Residual
347 effect of 10% bifenthrin wp on mosquitoes, and community acceptance, in eastern
348 Thailand. Southeast Asian J. Trop. Med. Public Health.40: 1221–1225.
- 349 Kunz, S., K. Murrell, G. Lambert, L. James, and C. Terrill. 1991. Estimated losses of
350 livestock to pests. J CRC handbook of pest management in agriculture. 1: 69–98.
- 351 Lorn, S., S. Ratisupakorn, G. Duvallet, T. Chareonviriyaphap, and K. Tainchum. 2020.
352 Species composition and abundance of *Stomoxys* spp. (Diptera: Muscidae) in
353 peninsular Thailand. J. Med. Entomol. 57: 252–258.
- 354 Marcon, P. C., G. D. Thomas, B. D. Siegfried, and J. B. Campbell. 1997. Susceptibility of
355 stable flies (Diptera: Muscidae) from southeastern Nebraska beef cattle feedlots to
356 selected insecticides and comparison of 3 bioassay techniques. J. Econ. Entomol. 90:
357 293–298.
- 358 Nkya, T. E., I. Akhouayri, W. Kisinza, and J. P. David. 2013. Impact of environment on
359 mosquito response to pyrethroid insecticides: facts, evidences and prospects. Insect
360 Biochem. Mol. Biol. 43. 407–416.

Lorn et al. 2021- Pyrethroid susceptibility of *Stomoxys* spp.

- 361 (ODOC5) Office of Prevention and Control 5 Ratchaburi. 2018. Fly control (Thai), Public
362 Health Department, Ministry of Public Health, Ratchaburi.
- 363 Olafson, P. U., P. E. Kaufman, G. Duvallet, J. A. Solórzano, D. B. Taylor, and R. T. Fryxell.
364 2019. Frequency of *kdr* and *kdr-his* alleles in stable Fly (Diptera: Muscidae)
365 populations from the United States, Costa Rica, France, and Thailand. J. Med.
366 Entomol. 56: 1145–1149.
- 367 (OVBDC) Office of Vector-Borne Disease Control. 1999. Malaria. Malaria Division,
368 Ministry of Public Health, Nonthaburi.
- 369 Pakvilai, N., T. Prapamontol, P. Thavornnyutikarn, A. Mangklabruks, S. Chantara, and C.
370 Santasup. 2012. Residues of synthetic pyrethroid pesticides in vegetables, fruit,
371 sediment and water from an intensive agricultural area (Fang district, Chiang Mai,
372 Thailand). WIT Trans. Ecol. Environ. 167: 201–210.
- 373 Pitzer, J. B., P. E. Kaufman, and S. H. Tenbroeck. 2010. Assessing permethrin resistance in
374 the stable fly (Diptera: Muscidae) in Florida by using laboratory selections and field
375 evaluations. J. Econ. Entomol. 103: 2258–2263.
- 376 Reissert-Oppermann, S., B. Bauer, S. Steuber, and P.-H. Clausen. 2019. Insecticide resistance
377 in stable flies (*Stomoxys calcitrans*) on dairy farms in Germany. Parasitol. Res. 118:
378 2499–2507.
- 379 Roche, X., A. Rozstalnyy, D. TagoPacheco, C. Pittiglio, A. Kamata, D. Beltran Alcrudo, K.
380 Bisht, S. Karki, J. Kayamori, F. Larfaoui, E. Raizman, S. VonDobschuetz, M.S.
381 Dhingra, and K. Sumption. 2020. Introduction and spread of lumpy skin disease in
382 South, East and Southeast Asia: Qualitative risk assessment and management. FAO
383 animal production and health, FAO, Rome.
- 384 Salem, A., E. Bouhsira, E. Lienard, A. B. Melou, P. Jacquet, and M. Franc. 2012.
385 Susceptibility of two European strains of *Stomoxys calcitrans* (L.) to cypermethrin,

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- 386 deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin and phoxim. Intern. J. Appl.
387 Res. Vet. Med. 10: 249–257.
- 388 Somme, L. 1958. The number of stable flies in norwegian barns, and their resistance to DDT.
389 J. Econ. Entomol. 51: 599–601.
- 390 Tainchum, K., S. Sukonthabhirom, G. Duvallet, P. Akratanakul, V. Muenworn, and T.
391 Chareonviriyaphap. 2010. Population structure of *Stomoxys calcitrans* (Diptera:
392 Muscidae) from nine regions of Thailand. J. Econ. Entomol. 103: 1012–1018.
- 393 Tainchum, K., S. Shukri, G. Duvallet, L. Etienne, and P. Jacquet. 2018. Phenotypic
394 susceptibility to pyrethroids and organophosphate of wild *Stomoxys calcitrans*
395 (Diptera: Muscidae) populations in southwestern France. Parasitol. Res. 117: 4027–
396 4032.
- 397 Taylor, D. B. and D.R. Berkebile. 2006. Comparative efficiency of six stable fly (Diptera:
398 Muscidae) traps. J. Econ. Entomol. 99: 1415–1419.
- 399 Taylor, D. B. and D. R. Berkebile. 2008. Sugar feeding in adult stable flies. Environ.
400 Entomol. 37: 625–629.
- 401 Taylor, D. B., R. D. Moon, and D. R. Mark. 2012. Economic impact of stable flies (Diptera:
402 Muscidae) on dairy and beef cattle production. J. Med. Entomol. 49: 198–209.
- 403 (WHO) World Health Organization. 2006. Guidelines for Testing Mosquito Adulticides for
404 Indoor Residual Spraying and Treatment of Mosquito Nets. World Health
405 Organization, Geneva.
- 406 (WHO) World Health Organization. 2016. Test Procedures for Insecticide Resistance
407 Monitoring in Malaria Vector Mosquitoes. World Health Organization, Geneva.
- 408 Wongta, A., N. Sawarng, P. Tongchai, K. Sutan, T. Kerdnoi, T. Prapamontol, and S.
409 Hongsibsong, 2018. The pesticide exposure of people living in agricultural
410 community, northern Thailand. J. Toxicol. 1: e4168034.

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411 **Table 1.** The collection sites of stable flies were located in three provinces: Songkhla (SON),
412 Phatthalung (PHA), and Satun (SAT). The associated characteristics and insecticide use are
413 summarized

Collection site	Reference	Characteristics
Point		
Songkhla	7°00'11.6"N	Dairy farm (nearby swine, poultry, and goat farm) located in
(SON)	100°30'15.4"E	Prince of Songkla University (PSU), Hat Yai District, Songkhla Province Organophosphate treatment
Phatthalung	7°48'13.1"N	Dairy farm located in Thaksin University (TSU), Pa Phayom
(PHA)	99°55'20.0"E	District, Phatthalung Province Pyrethroid treatment
Satun	6°55'49.2"N	Organic local cow farm located in Somyot Wechasi cattle
(SAT)	99°57'47.5"E	breeding farm, Udai Charoen, Khuan Kalong District, Satun Province Untreated site

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Table 2. Wild adult *Stomoxys calcitrans* (males and females) sampled from Songkhla (SON) and Phattalung (PHA) populations, the mean knockdown (KD) and mortality rates (percentage $\pm$ SE) of within 60 min and 24h following 30 min exposure to recommended concentrations of six technical grade and one commercial-grade insecticide, in WHO cone bioassay

Population	Insecticide [†]	Number Exposed (n)	Dead	Mean percent responses [*]		Status [‡]
				KD	Mortality	
SON	Permethrin 0.5500% (T)	31	23	96.67 $\pm$ 3.33a	73.89 $\pm$ 4.42ab	R
	Deltamethrin 0.0500%(T)	30	14	100.00 $\pm$ 0.00a	46.67 $\pm$ 8.43bc	R
	Alpha-cypermethrin 0.0750%(T)	30	10	90.00 $\pm$ 6.83a	33.33 $\pm$ 13.33cd	R
	Cypermethrin 0.2500%(T)	28	10	93.33 $\pm$ 4.22a	35.56 $\pm$ 6.13cd	R
	Lambda-cyhalothrin 0.0750%(T)	29	22	100.00 $\pm$ 0.00a	76.67 $\pm$ 9.54ab	R
	Bifenthrin 0.0625%(T)	28	27	80.83 $\pm$ 7.57a	95.83 $\pm$ 4.17a	I
	Cypermethrin 25.00%(C)	30	13	96.67 $\pm$ 3.33a	43.33 $\pm$ 6.15bc	R
	Control	30	1	0.00 $\pm$ 0.00b	3.33 $\pm$ 3.33d	-
	Permethrin 0.5500% (T)	30	22	83.33 $\pm$ 8.03a	73.33 $\pm$ 4.43ab	R
	Deltamethrin 0.0500%(T)	29	20	93.33 $\pm$ 6.67a	67.50 $\pm$ 9.11ab	R
PHA						

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Alpha-cypermethrin	30	13	93.33±4.22a	43.33±3.33cb	R
0.0750%(T)					
Cypermethrin	30	8	76.67±12.02a	26.67±4.22cd	R
0.2500%(T)					
Lambda-cyhalothrin	30	19	96.67±3.33a	63.33±8.03ab	R
0.0750%(T)					
Bifenthrin	29	25	73.33±15.20a	86.67±6.67a	R
0.0625%(T)					
Cypermethrin	30	13	100.00±0.00a	43.33±14.06bc	R
25.00%(C)					
Control	30	0	0.00±0.00b	0.00±0.00d	—

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420 * Means followed by the same letter(s) within a column are not significantly different at 5%

421 level of significance ($p \leq 0.05$) according to Tukey's comparison test,

422 [†]T: technical grade; C: commercial grade,

423 [‡]R: resistant to insecticide; I: incipient resistance to insecticide; S: susceptible to insecticide

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Table 3. Wild *Stomoxys indicus* (males and females) adults from Phattalung (PHA) and Satun (SAT) populations, the percent knockdown (KD) at 60 min and 24h mortality (percentage $\pm$ SE) after exposure to an insecticide at recommended concentration, for six technical grade and a commercial-grade insecticide

Population	Insecticide [†]	Number Exposed (n)	Dead	Mean percent responses [*]		Status [‡]
				KD	Mortality	
PHA	Permethrin 0.5500% (T)	30	29	100.00 $\pm$ 0.00a	96.67 $\pm$ 3.33a	I
	Deltamethrin 0.0500%(T)	30	8	76.67 $\pm$ 6.15ab	26.67 $\pm$ 8.43cd	R
	Alpha- cypermethrin 0.0750%(T)	30	8	73.33 $\pm$ 9.89ab	26.67 $\pm$ 8.43cd	R
	Cypermethrin 0.2500%(T)	31	17	76.19 $\pm$ 11.01ab	52.50 $\pm$ 14.01bc	R
	Lambda- cyhalothrin 0.0750%(T)	29	4	47.50 $\pm$ 11.95b	13.33 $\pm$ 6.67cd	R
	Bifenthrin 0.0625%(T)	30	21	63.33 $\pm$ 9.55ab	70.00 $\pm$ 15.28ab	R
	Cypermethrin 25.00%(C)	29	11	83.33 $\pm$ 8.03ab	38.33 $\pm$ 6.54bcd	R
	Control	29	1	0.00 $\pm$ 0.00c	3.33 $\pm$ 3.33d	–
	Permethrin 0.5500% (T)	31	31	100 $\pm$ 0.00a	100 $\pm$ 0.00a	S
	Deltamethrin	28	8	66.67 $\pm$ 13.33abc	29.17 $\pm$ 8.98bc	R

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0.0500%(T)					
Alpha-					
cypermethrin	28	14	96.67±3.33ab	49.17±10.68b	R
0.0750%(T)					
Cypermethrin	30	11	58.61±9.05bc	35.56±14.05bc	R
0.2500%(T)					
Lambda-					
cyhalothrin	30	12	40.00±10.33cd	40.00±5.16bc	R
0.0750%(T)					
Bifenthrin	28	15	46.67±14.59c	55.00±9.57b	R
0.0625%(T)					
Cypermethrin	30	13	60.00±7.30abc	43.33±9.55bc	R
25.00%(C)					
Control	27	2	0.00±0.00d	6.67±4.22c	—

430

431 * Means followed by the same letter(s) within a column are not significantly different at 5%

432 level of significance ($p \leq 0.05$) according to Tukey's comparison test,

433 † T: technical grade; C: commercial grade,

434 ‡ R: resistant to insecticide; I: incipient resistance to insecticide; S: susceptible to insecticide

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Table 4. Comparison of the mean number of pyrethroid insecticide response (mortality rates) of 4 populations of stable flies (1=*Stomoxys calcitrans* SON; 2= *Stomoxys calcitrans* PHA; 3= *Stomoxys indicus* PHA; 4= *Stomoxys indicus* SAT)

Chemical	Test populations*				Statistic
	1	2	3	4	
Permethrin 0.5500% (T)	73.33 ^b	73.33 ^b	73.33 ^b	100 ^a	$F=2.196$ $P=0.120$
Deltamethrin 0.0500%(T)	46.67 ^a ^b	67.50 ^a	67.50 ^a	29.17 ^b	$F=3.278$ $P=0.042$
Alpha-cypermethrin 0.0750%(T)	33.33 ^a	43.33 ^a	43.33 ^a	49.17 ^a	$F=0.317$ $P=0.813$
Cypermethrin 0.2500%(T)	35.55 ^a	26.67 ^a	26.67 ^a	35.55 ^a	$F=2.069$ $P=0.137$
Lambda-cyhalothrin 0.0750%(T)	76.67 ^a	63.33 ^{ab}	63.33 ^{ab}	40.00 ^b	$F=2.196$ $P=0.120$
Bifenthrin 0.0625%(T)	95.83 ^a	86.67 ^a	86.67 ^a	55.00 ^b	$F=1.230$ $P=0.325$
Cypermethrin 25.00%(C)	43.33 ^a	43.33 ^a	43.33 ^a	43.33 ^a	$F=23.621$ $P=0.000$
Control	3.33 ^a	0.00 ^a	0.00 ^a	6.67 ^a	$F=0.000$ $P=0.000$

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* Means followed by the same letter(s) within a row are not significantly different at 5% level of significance ($p \leq 0.05$) according to Tukey's comparison test.

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Figure legends

Fig 1. Collection sites of *Stomoxys calcitrans* and *Stomoxys indicus* were located in 3 provinces; Phatthalung, Satun, and Songkhla, Southern Thailand

Fig 2. The differential response of *Stomoxys calcitrans* (A: Songkhla, B: Phatthalung) *Stomoxys indicus* (C: Phatthalung, D: Satun) exposed to pyrethroids (per=permethrin, del=deltamethrin, alp=alphacypermethrin, cyt=cypermethrin (tec.), lam=lambdacyhalothrin, bif=bifenthrin, cyc=cypermethrin (com.)) at two knockdown observations (60 min and 30 min after exposure) and mortalities (24hr and 12hr), DMT: differential mortality time, DKT: differential knockdown time (DMT)

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Fig 1.

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Fig 2.