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**ORIGINAL ARTICLE**

# Susceptibility and Resistance of Field Populations of *Anopheles sinensis* (Diptera: Culicidae) Collected from Paju to 13 Insecticides

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**Abstract**

**Objectives:** Over 20% of all malaria cases reported annually in the Republic of Korea (ROK) occur in Paju, Gyeonggi Province. Vector control for malaria management is essential, but the insecticide resistance of the vector, *Anopheles* mosquitoes, has been a major obstacle in implementing effective control. In this study, the insecticide resistance of the vector mosquitoes was evaluated and compared with that of vector mosquitoes collected from the same locality in 2001 and 2009.

**Methods:** The insecticide resistance of *Anopheles sinensis* s.s. collected from Paju, Gyeonggi Province in the ROK was evaluated under laboratory conditions with a micro-application method using 13 insecticides currently used by local public health centers and pest control operators in the ROK.

**Results:** Based on median lethal dose ($LC_{50}$) values, *An. sinensis* s.s. were most susceptible to the insecticides bifenthrin, cyfluthrin, and etofenprox in that order, and least susceptible to permethrin. *An. sinensis* showed higher susceptibility to pyrethroids than organophosphates, except for fenthion and permethrin. In a comparative resistance test, the resistance ratios (RRs) of *An. sinensis* collected in 2012 (AS12) to the 13 insecticides were compared to the RRs of two strains of *An. sinensis* collected from the same locality in 2001 (AS01) and 2008 (AS08). With some exceptions, AS12 demonstrated higher resistance to all tested insecticides compared to AS01 and AS08, and less resistance to bifenthrin, cyfluthrin, and cypermethrin compared to AS01.

**Conclusion:** These results indicate that careful selection and rotation of these insecticides may result in continued satisfactory control of field populations of *An. sinensis* s.s. for effective malaria management in Paju.

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## 1. Introduction

In the Republic of Korea (ROK, South Korea), *Plasmodium vivax* malaria has been endemic for centuries [1,2]. In the 1960s and 1970s, active and passive uses of pesticides were combined in an ambitious eradication project by the ROK government [1]. In the mid-1970s, indigenous transmission of malaria was greatly reduced and the ROK was declared a "malaria free zone" by the World Health Organization (WHO) [3,4]. However, *Plasmodium vivax* re-emerged in Gyeonggi province in 1993. Subsequently, vivax malaria increased to 4412 cases in 2000, before declining to 864 cases in 2004, rising in 2005 [5,6], and again decreasing to 843 cases in 2011 [7]. Since 2001 over 20% of all reported vivax malaria cases have occurred Paju, Gyeonggi Province, which highlights the necessity of vector control in that locality. The vector mosquito is one of the most important factors in the transmission of vivax malaria and therefore must be effectively controlled. To protect public health, the susceptibility and resistance of vector populations to registered insecticides need to be monitored so that effective control measures can be implemented [8].

The principal measures to control mosquito populations include the use of various contact residual insecticides, e.g., organophosphates, carbamates, and pyrethroids. However, repeated use can result in the development of resistance to these insecticides [9—13]. Also, as insects become resistant, more insecticides may be applied, resulting in human and environmental health problems. Widespread insecticide resistance to commonly used and less expensive insecticides has been a major obstacle to implementing cost-effective and safe integrated mosquito management programs. In addition, the use of certain insecticides will likely be reduced in the near future in the USA by the U.S. Environmental Protection Agency (EPA) under the 1996 Food Quality and Protection Act [14].

These problems indicate the need to establish effective insecticide resistance management strategies, which include the collection of baseline data and determination of insecticide resistance trends. This study evaluated the susceptibility of *An. sinensis* collected in Paju in 2012 to 13 commonly used insecticides in the ROK and monitored changes in insecticide resistance compared to the same species collected in the same locality in 2001 and 2009.

## 2. Materials and Methods

### 2.1. Chemicals

Thirteen different insecticides purchased from Fluka (Buchs, Switzerland) were used in this study: bifenthrin (97.0% purity), cyfluthrin (93.0%), etofenprox (96.5%), fenthion (95.5%), cypermethrin (98.0%), λ-cyhalothrin (98.6%), α-cypermethrin (97.5%), deltamethrin (99.5%), dichlorvos (99.5%), chlorpyrifos (98.5%), fenitrothion (98.5%), profenofos (98.0%), and permethrin (95.5%). Triton X-100 was obtained from Shinoy Pure Chemicals (Osaka, Japan). All other chemicals used were of analytical grade and available commercially.

### 2.2. Mosquitoes

Engorged female mosquitoes were collected using black light traps (Yoshizawa type, FL 6w; Shinyoung Co., Seoul, Korea) and an aspirator at cow sheds in Tong Il Chon, Baegyeon, Paju, Gyeonggi Province from July to August, 2012. To induce oviposition, engorged females were placed individually in paper cups (350 mL) lined with filter papers and half filled with distilled water.

The eggs were allowed to hatch in larval rearing pans (15 × 15 × 4 cm). The larvae were provided with a mixture of Vivid-S (Sewhapet Co., Incheon, Korea) and Super Terramin (Sewhapet Co.) which was sprinkled over the surface of the water. Larvae were reared in an insectary at 25 ± 1 °C, with 65 ± 5% relative humidity and a photoperiod of 14 hours light:16 hours dark. The identification of field collected populations was confirmed by polymerase chain reaction (PCR) [15,16].

### 2.3. Mosquito identification

The identity of the *Anopheles* species was confirmed by PCR with genomic DNA extracted from the legs of individual adult mosquitoes. The PCR products were separated on a 2% agarose gel and visualized with Safe-Pinky DNA Gel staining solution (×10,000) (GenDEPOT, Barker, TX, USA). Fragment sizes were estimated by comparison to molecular weight standards provided by a 100-bp Ladder Molecular Weight DNA Marker (Bioneer, Seoul, ROK) (Table 1).

### 2.4. Bioassay

A direct-contact mortality bioassay [4] was used to evaluate the toxicity of 15 larvicides to late third instars of *An. sinensis* s.s. from the field-collected colonies. Each larvicide was dissolved in methanol and then further diluted in distilled water containing Triton X-100 (20 μL/l). A total of 25 larvae from each colony were placed in paper cups (350 mL) containing test larvicide solutions (250 mL). The toxicity of each test larvicide was determined using four to six concentrations ranging from 1 ppm to 200 ppm. The control consisted of the methanol—Triton X-100 carrier solution in distilled water. Treated and control groups were held under the same conditions as used for colony maintenance.

Larvae were considered to be dead if they did not move when they were prodded with a fine wooden dowel 24 hours after treatment [17]. All treatments were replicated three times using 25 larvae/replicate. Because bioassays could not all be conducted simultaneously, treatments were blocked over time with a separate

**Table 1.** Primers and PCR conditions for the identification of seven *Anopheles* spp.

| Primer | Species | Sequence (5’→3’) | Diagnostic bands |
|---|---|---|---|
| Forward | Universal primer | TGT GAA CTG CAG GAC ACA TGA A | — |
| Reverse | *Anopheles sinensis* | ATT GTT GTC CAG CCC GCT AAC | 500 bp |
| | *Anopheles pulls* | ATA TCA TGG CTT AAC ACC GCG T | 260 bp |
| | *Anopheles lesteri* | TGC CTA GAA CTT CCG CCA ATC | 300 bp |
| | *Anopheles belenrae* | CAT TTT TCA CGA CTG CGA CGG | 190 bp |
| | *Anopheles kleini* | GCG TCC ATA CTG TCT CAA CGA | 400 bp |
| | *Anopheles sineroides* | ACC GAG TGG CCT CAC TC | 500 bp |
| | *Anopheles koreicus* | GTA TAC ACG CTT TGT ATG TGG GG | 200 bp |

| Primer | Reagent | Volume |
|---|---|---|
| Reaction mixture | PCR premix kit (Accupower, Bioneer) | — |
| | Primer (each 10 pmol/μL) | 3.0 μL (each 0.5 μL) |
| | DNA template (10 ng/μL) | 2.0 μL |
| | Triple deionized distilled water | 15.0 μL |
| | Total | 20.0 μL |

| Primer | Temperature (°C) | Time (min) | Cycles |
|---|---|---|---|
| PCR | 94 | 10 | 1 |
| | 94 | 0.5 | 34 |
| | 60 | 1 | |
| | 72 | 1 | |
| | 72 | 5 | 1 |
| | 4 | ∞ | ∞ |

control treatment used for each block. Freshly prepared solutions were used for each block of bioassays [18].

### 2.5. Data analysis

Data were corrected for mortality using Abbott’s formula [19]. Mortality rates were analyzed using a probit analysis with SAS software (SAS, Cary, NC, USA). The resistance ratio (RR), defined as the ratio produced when the 50% mortality ($LC_{50}$) values of the strain collected in 2012 (AS12) were divided by the $LC_{50}$ values reported for mosquitoes tested in 2001 (AS01) and 2008 (AS08), was used as described by Shin et al [11].

The RRs were used to compare the susceptibility of larvae of field-collected *An. sinensis* collected and assayed in 2001, 2008, and 2012. RRs values of <10, 10−40, 40−160, and >160 were classified as low, moderate, high, and extremely high resistance, respectively [20]. The $LC_{50}$ values of the treatments were considered to be significantly different from one another when their 95% confidence limits (CL) failed to overlap.

## 3. Results

The $LC_{50}$ values demonstrated that the susceptibility of the larvae of *An. sinensis* s.s. collected from Paju in 2012 (AS12) was highest to bifenthrin, followed by cyfluthrin, etofenprox, fenthion, cypermethrin, λ-cyhalothrin, α-cypermethrin, deltamethrin, dichlorvos, chlorpyrifos, profenofos, fenitrothion, and permethrin, in that order (Table 2). AS12 showed the highest susceptibility to bifenthrin with $LC_{50}$ found at 0.227 ppm, followed by cyfluthrin and etofenprox with $LC_{50}$ at 0.446 ppm and 1.858 ppm, respectively, and demonstrated the lowest susceptibility to permethrin with $LC_{50}$ at 12.485 ppm. AS12 exhibited a 50-fold lower susceptibility to permethrin than to bifenthrin. AS12 showed higher susceptibility to pyrethroids than organophosphates, except for fenthion and permethrin.

Comparative analysis of data for larvae collected from the same locality in 2001 (AS01) and 2008 (AS08) was carried out for the 13 insecticides [10, 21] (Table 3). Chang et al [10] showed that AS08 exhibited decreased pyrethroid resistance compared to AS01, except for permethrin and deltamethrin. The $RR_{08\text{-}01}$ values of *An. sinensis* to pyrethroids ranged from 0.03 to 0.40 as follows: bifenthrin: 0.03; λ-cyhalothrin: 0.06; α-cypermethrin: 0.30; cypermethrin: 0.34; and cyfluthrin: 0.40. The $RR_{08\text{-}01}$ values of deltamethrin and permethrin were 1.50 and 3.88 (low resistance level), respectively. However, AS12 showed higher pyrethroid resistance than AS08, with $RR_{12\text{-}08}$ values ranging from 15.07 to 55.38 (moderate to high) as follows: α-cypermethrin: 55.38; λ-cyhalothrin: 40.25; bifenthrin: 25.22; and deltamethrin: 15.07. The $RR_{12\text{-}08}$ values of *An. sinensis* to cypermethrin, cyfluthrin, and permethrin were less than 10-fold greater.

The resistance of *An. sinensis* to five organophosphates has continuously increased since 2001.

The $RR_{08\text{-}01}$ of *An. sinensis* to organophosphates were low to moderate with values of 1.38 ppm to

**Table 2.** Susceptibility of 13 insecticides against *Anopheles sinensis* s.s. using direct contact diffusion assay with 24-hour exposure

| Insecticide | *n*[a] | Slope (±SE) | $LC_{50}$ (ppm) | 95% CL | $\chi^2$ | RS[b] |
|---|---|---|---|---|---|---|
| Bifenthrin | 450 | 1.0 ± 0.09 | 0.227 | 0.166−0.312 | 5.35 | 1.0 |
| Cyfluthrin | 375 | 1.0 ± 0.12 | 0.446 | 0.315−0.692 | 4.69 | 2.0 |
| Etofenprox | 450 | 1.0 ± 0.90 | 1.858 | 1.360−2.331 | 6.53 | 8.2 |
| Fenthion | 300 | 1.3 ± 0.16 | 2.377 | 1.732−3.120 | 2.65 | 10.5 |
| Cypermethrin | 375 | 1.0 ± 1.12 | 2.419 | 1.748−3.217 | 1.83 | 10.7 |
| λ-Cyhalothrin | 300 | 1.2 ± 0.15 | 3.220 | 2.410−3.543 | 0.54 | 14.2 |
| α-Cypermethrin | 300 | 1.4 ± 0.16 | 3.323 | 2.561−3.689 | 2.70 | 14.6 |
| Deltamethrin | 375 | 1.4 ± 0.14 | 4.522 | 3.444−5.216 | 6.03 | 19.9 |
| Dichlorvos | 450 | 1.1 ± 0.13 | 4.850 | 3.907−5.224 | 3.57 | 21.4 |
| Chlorpyrifos | 375 | 1.2 ± 0.13 | 4.890 | 3.951−5.336 | 3.39 | 21.5 |
| Profenofos | 375 | 1.6 ± 0.14 | 5.829 | 4.916−7.361 | 3.89 | 25.7 |
| Fenitrothion | 450 | 1.3 ± 0.13 | 7.860 | 6.115−9.124 | 2.99 | 34.6 |
| Permethrin | 375 | 1.5 ± 0.16 | 12.485 | 9.799−13.599 | 0.45 | 55.0 |

[a] *Number of mosquitoes;* [b] *$LC_{50}$ of each insecticide/$LC_{50}$ of bifenthrin.* CL = confident limit; $LC_{50}$ = median lethal dose; RS = relative susceptibility.

36.67 ppm. AS08 showed moderate levels of resistance to fenthion and profenofos with RR values of 36.67 and 12.33, and low levels of resistance to dichlorvos, fenitrothion, and chlorpyrifos with RR values of 1.84 ppm, 1.54 ppm, and 1.38 ppm, respectively. When compared to AS01, the $RR_{12\text{-}08}$ of *An. sinensis* to organophosphates were low with values ranging from 1.02 to 2.16, as follows: fenitrothion: 1.02; chlorpyrifos: 1.04; dichlorvos: 1.39; profenofos: 1.58; and fenthion: 2.16. Although the organophosphate resistance level of AS08 was low, resistance to organophosphates had not decreased. The $RR_{12\text{-}01}$ of *An. sinensis* were low to high with values of 1.44 to 79.23. AS12 to fenthion demonstrated a high level of resistance with an RR value of 79.23, a moderate level of resistance to profenofos with an RR value of 19.43, and low levels of resistance to dichlorvos, fenitrothion, and chlorpyrifos with RR values of 2.55, 1.57, and 1.44, respectively.

## 4. Discussion

Insecticides have played a major role in the control of agricultural pests and vectors in the ROK, but their long and frequent use has resulted in significant insecticide resistance [8,10−13]. In our study, *An. sinensis* s.s. collected in 2012 showed high levels of pyrethroid resistance compared to samples gathered from the same locality in 2008.

**Table 3.** Comparison of insecticide susceptibility of larvae of *Anopheles sinensis* s.s. among 2001 [21], 2008 [10], and 2012 strains collected in Paju, Republic of Korea

| | $LC_{50}$ (ppm) | | | | | |
|---|---|---|---|---|---|---|
| Insecticide | AS01[a] | AS08 | AS12 | $RR_{12\text{-}01}$[b] | $RR_{12\text{-}08}$ | $RR_{08\text{-}01}$ |
| **Organophosphates** | | | | | | |
| Fenthion | 0.03 | 1.1 | 2.377 | 79.23 | 2.16 | 36.67 |
| Dichlorvos | 1.9 | 3.5 | 4.850 | 2.55 | 1.39 | 1.84 |
| Chlorpyrifos | 3.4 | 4.7 | 4.890 | 1.44 | 1.04 | 1.38 |
| Profenofos | 0.3 | 3.7 | 5.829 | 19.43 | 1.58 | 12.33 |
| Fenitrothion | 5.0 | 7.7 | 7.860 | 1.57 | 1.02 | 1.54 |
| **Pyrethroids** | | | | | | |
| Bifenthrin | 0.28 | 0.009 | 0.227 | 0.81 | 25.22 | 0.03 |
| Cyfluthrin | 0.5 | 0.2 | 0.446 | 0.89 | 2.23 | 0.40 |
| Cypermethrin | 4.7 | 1.6 | 2.419 | 0.51 | 1.51 | 0.34 |
| λ-Cyhalothrin | 1.3 | 0.08 | 3.220 | 2.48 | 40.25 | 0.06 |
| α-Cypermethrin | 0.2 | 0.06 | 3.323 | 16.62 | 55.38 | 0.30 |
| Deltamethrin | 0.2 | 0.3 | 4.522 | 22.61 | 15.07 | 1.50 |
| Permethrin | 0.8 | 3.1 | 12.485 | 15.61 | 4.03 | 3.88 |

[a] *AS01, colony of Shin et al, 2003 [21]; AS08, colony of Chang et al, 2009 [10]; and AS12, colony collected in 2012;* [b] *$RR_{12\text{-}01}$ stands for $LC_{50}$ of AS08/$LC_{50}$ of AS01; $RR_{12\text{-}08}$, $LC_{50}$ of AS12/$LC_{50}$ of AS08; and $RR_{08\text{-}01}$ $LC_{50}$ of AS08/$LC_{50}$ of AS01.* $LC_{50}$ = median lethal dose; RR = resistance ratio.

These findings may be the result of increased use of pyrethroids for agricultural pest control. According to pest control operators in this area, they changed to pyrethroids in 2007 because organophosphates were failing to control agricultural pests . Because *An. sinensis* breeds mainly in paddy fields, it is under heavy selection pressure due to the agricultural application of insecticides [10,21]. Pyrethroids have also been used for thermal fogging, residual spraying, and as a repellent applied to clothing at low concentrations for medical pests. These uses may have resulted in the development of resistance to pyrethroids over 4 years. Although the use of organophosphates against agricultural pests has decreased in this area since 2007 and resistance to organophosphates is now low, constant use may maintain the same level of resistance of *An. sinensis*. *An. sinensis* collected in 2008 showed higher resistance to organophosphates than pyrethroids compared to the sample collected in 2001, because organophosphate insecticides have been used primarily to control agricultural pests from 2001 to 2007 [10].

Resistance monitoring is an effective component of resistance management as it provides current information on the response of *An. sinensis* populations to insecticides. Susceptibility tests need to be conducted over a broad area, as insecticide pressures and usage may very geographically. Insecticide failures in the ROK have probably occurred as a result of the development of field resistance [9−11,13,22]. Early detection of trends in the development of potential resistance can facilitate the use of synergists, rotation of insecticides and/or classes of insecticides, and alternative technologies that reduce dependence on and usage of chemical insecticides [13,23,24].

These results indicate that strategies which limit insecticide use and discourage it when no longer effective, and encourage the selective rotation of classes of insecticides provide increased vector control against field populations of the malaria vector, *An. sinensis*, in the ROK.

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## References

1. Paik YK, Ree HI, Shim JC. Malaria in Korea. Jpn J Exp Med 1988 Mar;58(2):55−66.
2. Jun G, Yeom JS, Hong JY, et al. Resurgence of *Plasmodium vivax* malaria in the Republic of Korea during 2006−2007. Am J Trop Med Hyg 2009 Oct;81(4):605−10.
3. Ree HI. Unstable vivax malaria in Korea. Korean J Parasitol 2000 Sep;38(3):119−38.
4. WHO. Synopsis of the world malaria situation in 1979. Wkly Epidemiol Rec 1981;56:145−9.
5. Chai IH, Lim GI, Yoon SN, et al. Occurrence of tertian malaria in a male patient who has never been abroad. Korean J Parasitol 1994 Sep;32(3):195−200.
6. Lee WJ, Klein TA, Kim HC, et al. *Anopheles kleini*, *Anopheles pullus*, and *Anopheles sinensis*: potential vectors of *Plasmodium vivax* in the Republic of Korea. J Med Entomol 2007 Nov;44(6): 1086−90.
7. KCDC. Disease web statistics system. Available from: http://cdc.go.kr/kcdchome/ jsp/observation/stat/rgt/STATRGT0003List.jsp; 2012 [accessed 20.08.12].
8. Shin EH, Kim NJ, Kim HK, et al. Resistance of field-collected populations of *Culex pipiens pallens* (Diptera: Culicidae) to insecticides in the Republic of Korea. J Asia Pac Entomol 2012 Mar;15(1):1−4.
9. Kim NJ, Chang KS, Lee WJ, et al. Monitoring of insecticide resistance in field-collected populations of *Culex pipiens pallens* (Diptera: Culicidae). J Asia Pac Entomol 2007 Sep;10(3):1−5.
10. Chang KS, Jung JS, Park C, et al. Insecticide susceptibility and resistance of larvae of *Anopheles sinensis* group (Diptera: Culicidae) from Paju, Republic of Korea. Entomol Res 2009 May; 39(3):196−200.
11. Shin EH, Kim HK, Park C, et al. Insecticide susceptibility and resistance of *Culex tritaeniorhynchus* (Diptera: Culicidae) larvae collected from Gwangju, Republic of Korea. Entomol Res 2011 Jul;41(4):157−60.
12. Shin EH, Park C, Kim HK, et al. Insecticide susceptibility of *Ephemera orientalis* (Ephemeroptera: Ephemeridae) and two mosquito species, *Anopheles sinensis* and *Culex pipiens* in the Republic of Korea. J Asia Pac Entomol 2011 Sep;14(3):233−6.
13. Shin EH, Kim NJ, Kim HK, et al. Resistance of field-collected populations of *Culex pipiens pallens* (Diptera: Culicidae) to insecticides in the Republic of Korea. J Asia Pac Entomol 2012 Mar;15(1):1−4.
14. U.S. EPA. Pesticides: reregistration. Available from:. United States Environmental Protection Agency http://www.epa.gov/pesticides/reregistration/status.htm; 2010 [accessed 30.05.12].
15. Wilkerson RC, Li C, Rueda LM, et al. Molecular confirmation of *Anopheles (Anopheles) lesteri* from the Republic of South Korea and its genetic identity with *An.* (*Ano.*) *anthropophagus* from China (Diptera: Culicidae). Zootaxa 2003 Dec;378:1−14.
16. Li C, Lee JS, Groebner JL, et al. A newly recognized species in the *Anopheles* hyrcanus group and molecular identification of related species from the Republic of (South) Korea (Diptera: Culicidae). Zootaxa 2005 Apr;939:1−8.
17. Perumalsamy H, Kim NJ, Ahn YJ. Larvicidal activity of compounds isolated from *Asarum heterotropoides* against *Culex pipiens pallens, Aedes aegypti*, and *Ochlerotatus togoi* (Diptera: Culicidae). J Med Entomol 2009 Nov;46(6):1420−3.
18. Robertson JL, Preisler HK. Pesticide bioassays with arthropods. Boca Raton, FL, USA: CRC; 1992127.
19. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925 Jun;18(2):265−7.
20. Kim YJ, Lee YJ, Kim GH, et al. Toxicity of tebufenpyrad to *Tetranychus urticae* (Acari: Tetranychidae) and *Amblyseius wormersleyi* (Acari: Phytoseiidae) under laboratory and field conditions. J Econ Entomol 1999 Feb;92(1):187−92.
21. Shin EH, Park YI, Lee HI, et al. Insecticide susceptibilities of *Anopheles sinensis* (Dipteral: Culicidae) larvae from Paju-shi, Korea. Korean J Entomol 2003 Mar;33(1):33−7.
22. Shim JC, Hong HK, Lee DK. Susceptibilities of *Culex tritaeniorhynchus* larvae (Culicdae: Diptera) to insecticides. Korean J Entomol 1995 Jan;25(1):13−20.
23. Yeima M, Gwinn TA, Williams DC, et al. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo, Dominican Republic. J Am Mosq Contol Assoc 1991 Mar;7(1):69−72.
24. Lee DK, Shin EH, Shim JC. Insecticide susceptibility of *Culex pipiens pallens* (Culicidae, Diptera) larvae in Seoul. Korean J Entomol 1997 Mar;27(1):9−13.