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ARTICLE

Occurrence and insecticide susceptibility of the grain aphid *Sitobion avenae* in northern Mongolia

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Abstract: A survey was carried out to determine the prevalence of *Sitobion avenae* (*S. avenae*) in the central agriculture zone of northern Mongolia (including Darkhan-Uul, Selenge, Tuv, and Erdenet *aimags*). Bioassays were used to assess the pest's susceptibility to three regularly used insecticides (deltamethrin, fenvalerate, and imidacloprid). DNA sequencing was used to look into the genotypes of codons 918 and 1014 of the *Voltage-gated sodium channel* (*VGSC*) gene in *S. avenae*. The findings revealed that in 2019, the population density of *S. avenae* was low in 159 fields and medium in 31, and the infestation rate was low in 73 fields, medium in 117 fields. Even at a concentration of 0.005%, high mortality rates (>84 per cent) of *S. avenae* nymphs were detected after 72 hours of exposure to the three insecticides, indicating that this pest was susceptible to all three insecticides tested. In our grain aphid samples, the pyrethroid resistance-causing mutations (M918L and L1014F) in the voltage-gated sodium channel were not detected.

Keywords: *Sitobion avenae; infestation; insecticide susceptibility; population density; voltage-gated sodium channel;*

INTRODUCTION

In Europe, Asia and other parts of the world, aphid *Sitobion avenae* Fabricius (Hemiptera: Aphididae) is an important pest of cereal crops [1][2]. It is used in many important crops, including barley, wheat, oats, corn, sorghum, and rye. In addition to direct feeding, it also damages crops by transmitting plant viruses, including barley yellow dwarf virus [3].

Insecticides are still an essential and commonly used control method for *S. avenae*. Pyrethroids, such as lambda-cyhalothrin, cypermethrin, and deltamethrin, have all been shown to effectively suppress vulnerable S. avenae clones [4]. Theoretically, widespread pesticide usage could lead to resistance development.

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Indeed, insecticide resistance cases have been increasingly found in populations of *S. avenae*. For example, a recent study reported grain aphid populations exhibiting a high level of resistance to beta-cypermethrin (resistance ratio = 136.8) or to bifenthrin (resistance ratio = 313.4) [5]. Notably, spray failures with the above-mentioned compounds were first detected on cereal crops in England a decade ago (in 2011), and resistant aphid populations were found to contain individuals carrying the classic *kdr* (knockdown resistance) mutation (L1014F) in their *Voltage-gated sodium channel* (VGSC) gene, which affects pyrethroid binding and allows *S. avenae* to survive insecticide exposure [6][3][7][8]. The proportion of the clone carrying this mutation increased in the *S. avenae* populations from 2009 to 2014, and this clone was also detected in Ireland in 2013 [3], [7]–[10].

For a long-term pest control, it's critical to keep track of the population dynamics and the state of pesticide resistance in field populations. The *S. avenae* is a dominant species of grain aphids in northern Mongolia [11]. However, to our knowledge, there is no recent survey in Mongolia on its infestation and pesticide susceptibility. The aim of this study is to investigate (1) the population density and infestation rate of the *S. avenae*; (2) the status of insecticide susceptibility; and (3) the possible presence of *kdr* mutations in *S. avenae* populations in northern Mongolia.

MATERIALS AND METHODS

Aphid collection

The aphid collection was carried out throughout the major wheat-producing fields of Mongolia in 2019. For a total of 38 days, 190 wheat fields were surveyed along the transects of roughly 3915 km in Selenge, Darkhan-Uul, Tuv, and Erdenet aimags. The survey was conducted in the northern parts of Mongolia, which were mostly grain-growing regions. Net sweeping method was used to collect aphid samples from different geographic locations. Figure 1 shows the location of the study area.

Samples collected from the field were preserved in 100% ethanol for further laboratory analysis. The aphid specimens were identified morphologically and molecularly based on the *Mitochondrial cytochrome oxidase subunit I* (COI) gene as described in [12].

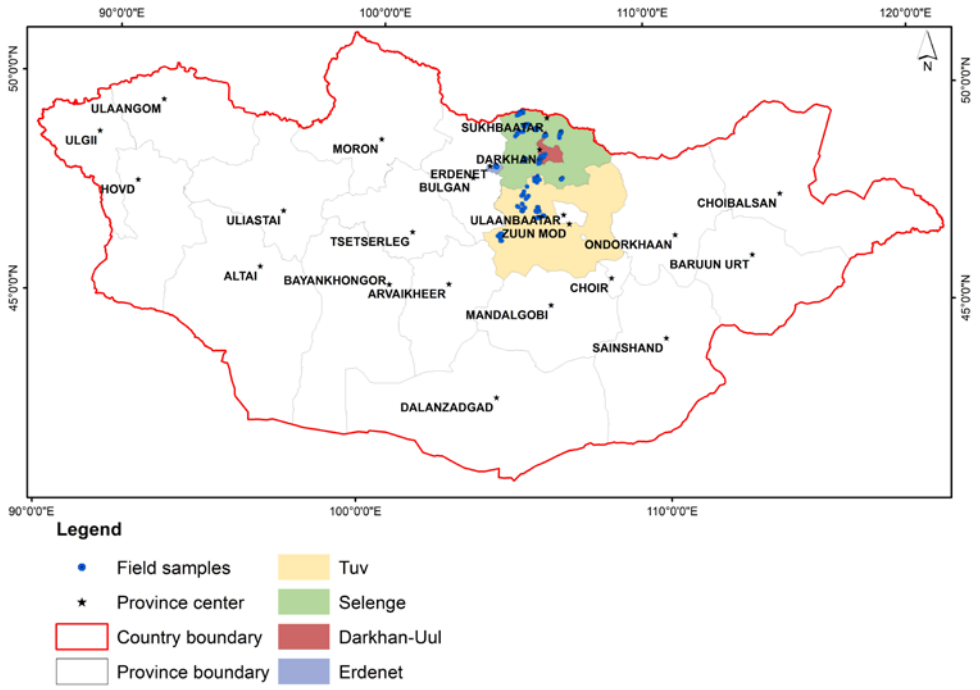

Figure 1. Sampling locations of Sitobion avenae in agricultural regions of Darkhan-Uul and Tuv aimags. The base map (Mongolia 1:2,500,000) was used for the analysis

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Estimate of population density and infestation

Aphid population density was graded on a scale of 1 to 3, where scale 1 means 1 to 10 individuals, scale 2 indicates 11 to 100 individuals, and scale 3 stands for more than 100 individuals per plant [13]. The infestation rate was evaluated by inspecting 50 plants within each field. It was ranked low if the infested plants were 1 to 10%, medium 11 to 30%, high 31 to 50%, and very high 51 to 100% [13].

Insecticide susceptibility bioassay

Insecticide susceptibility bioassays were carried out using 3rd instar nymph of grain aphids collected from Selenge and Darkhan-Uul aimags, in keeping with the protocol provided by the Insecticide Resistance Action Committee [14], [15]. In this assay, an average of three experimental replications are used with 6 sample replications. Mortality was recorded 72 hours after exposure to deltamethrin, fenvalerate, and imidacloprid at five different concentrations (i. e. 1%, 0.125%, 0.1%, 0.01%, 0.005%), with acetone as the only control (Figure 2). The aphids that survived exposure to pyrethroids (deltamethrin and fenvalerate) (namely pyrethroid surivivor) were kept for vgsc genotyping.

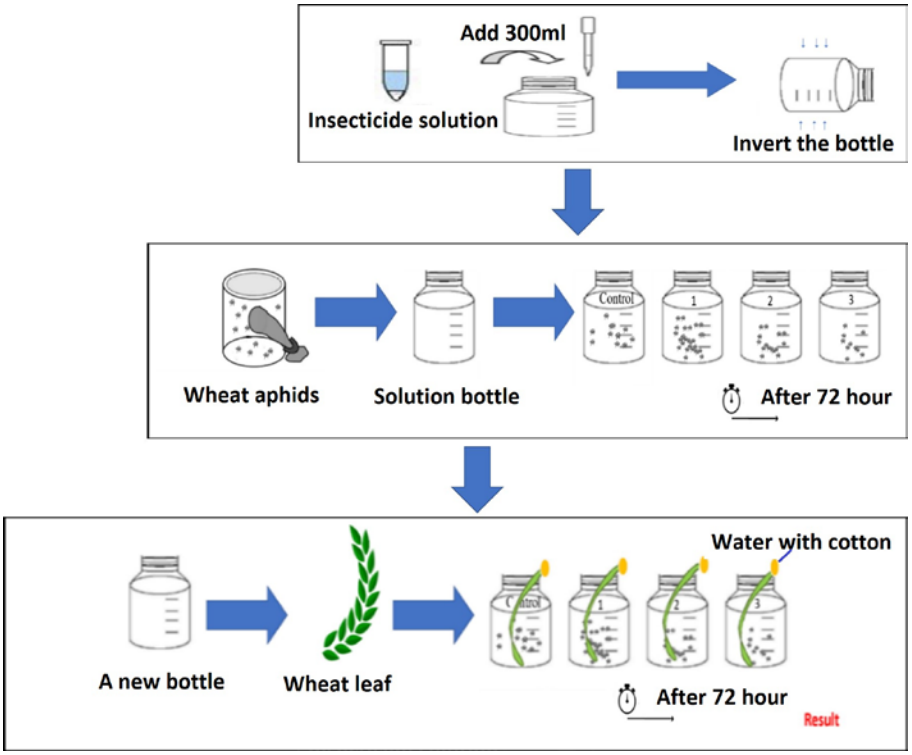

Figure 2. Scheme on insecticide exposure to S. avenae and assessment of mortality based on the methodology regulated by Insecticide Resistance Action Committee

Genotyping of the *Sitobion avenae* sodium channel gene

A fragment of the voltage-gated sodium channel gene containing codons 918 and 1014 was amplified using primers wakdr-F (5'-GACTGTCCGTATTACGTTCGT-3') and wakdr-R (5'-ATGTTGCCACCCCTAAATT CCT-3'). The PCR mixture in a total volume of 30 µl, consisted of 15 µl of 2 x Taq MASTER MIX (Tiangen Co., Beijing), 0.6 µl 10 µmoll from each primer, 12.8 µl of ddH_2O, and 1 µl of gDNA template. The thermal cycling profile included an initial step of denaturation at 95°C for 3 min followed by 38 cycles of 95°C for 30s, 55°C for 30s, and a final extension step at 72 °C for 5 min. The PCR products were gel-purified and directly Sanger sequenced using the forward primer wakdr-F.

Sequence analysis of vgsc fragments

DNA sequences obtained from DNA Sanger sequencing were manually trimmed and verified. Muscle 3.8 (Edgar, 2004) was used to align all confirmed DNA sequences, and polymorphism sites were recorded.

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RESULTS AND DISCUSSION

Population density and percentage infestation of *S. avenae*

In 159 fields, the population density of *S. avenae* was low (1 to 10 individuals per plant), while in 31 fields it was medium (11 to 100 per plant). The percentage of affected plants was low in 73 fields, while it was medium in 117 fields (Figure 3).

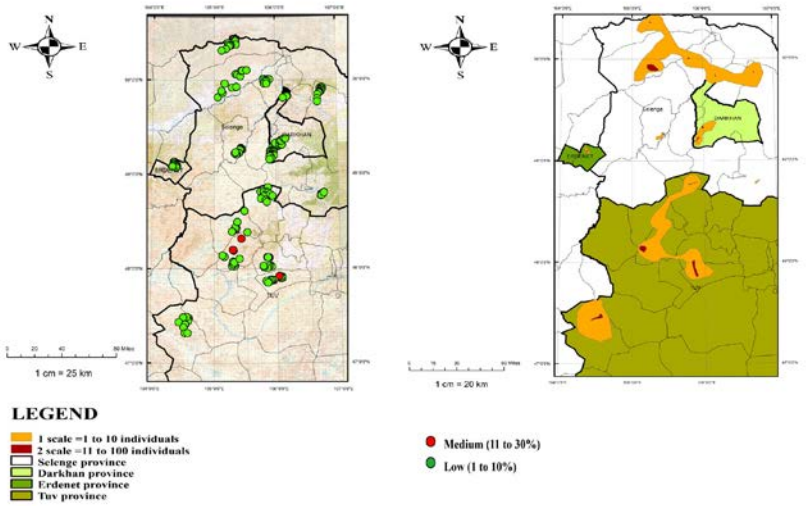

Figure 3. A. The population density, and B. Infestation rate of the Sitobion avenae in Mongolia. ArcGIS program was used to produce a distribution map based on the geographical coordinates of the sampling sites

These findings were similar to the Research report made by Malloch et al in UK between 2013 and 2015. The main difference was that suction traps are used to collect the aphid sample and samples are further used for measuring the population diversity for kdr and microsatellite genotype [9], [10].

Insecticide susceptibility

The bioassay results showed that the grain aphid, *S. avenae* is susceptible to the three tested pesticides. Under laboratory circumstances, all three pesticides resulted in high mortality rate of 84 to 100%. Even at the lowest concentration (0.005%), a significant mortality rate of >84% was recorded (Figure 4).

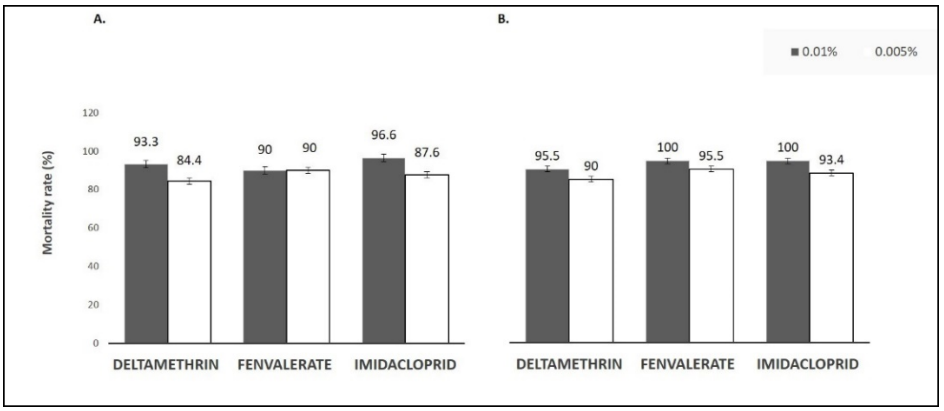

Figure 4. Mean mortality of Sitobion avenae after exposure to insecticides at different doses according to A. test No.019 and B. test No.030 of IRAC susceptibility test method series. Data shown are mean ± SE (n = 6)

Based on the analysis conducted by Malloch et al, *S. avenae* species were more resistant to insecticides belonging to pyrethroid family [9]. These differences in the findings are perhaps possibly due to a climate, ecology or pyrethroid selection pressure affecting this species.

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Genotyping of *S. avenae* vgsc

A total of 173 individuals of S. avenae, collected from different locations in Mongolia, as well as 42 survivors, were genotyped to detect the presence of either kdr or super kdr mutation (Table 1).

Table 1. Genotypes of the M918 mutation in the sodium channel of Sitobion avenae (Glover) collected from different wheat planting regions of Mongolia

Sampling site	Codon	918 AA	Frequency (%)	Codon	1014 Aa	Frequency (100%)	N
Darkhan	ATG	M	100	CTC	L	100	26
Selenge	ATG	M	100	CTC	L	100	84
Erdene	ATG	M	100	CTC	L	100	20
Tuv	ATG	M	100	CTC	L	100	60

Fragments of the VGSC gene, with a size of around 658 bp (Figure 5A), were sequenced.

DNA sequence analysis showed that the codons corresponding to amino acid at position 918 and 1014 were ATG and CTC respectively (Figure 5B), and all of the individuals tested were wild homozygotes at both codons (Table 1).

In 2015, *S. avenae* kdr genotypes were studied on the territories of England and Scotland. In this study, allelic discrimination PCR diagnostic test was used to detect the presence of the kdr mutation. As a result, highest kdr mutations were found in York and Preston areas, while no mutation was detected at Ayr, UK which corresponded with our findings. [10].

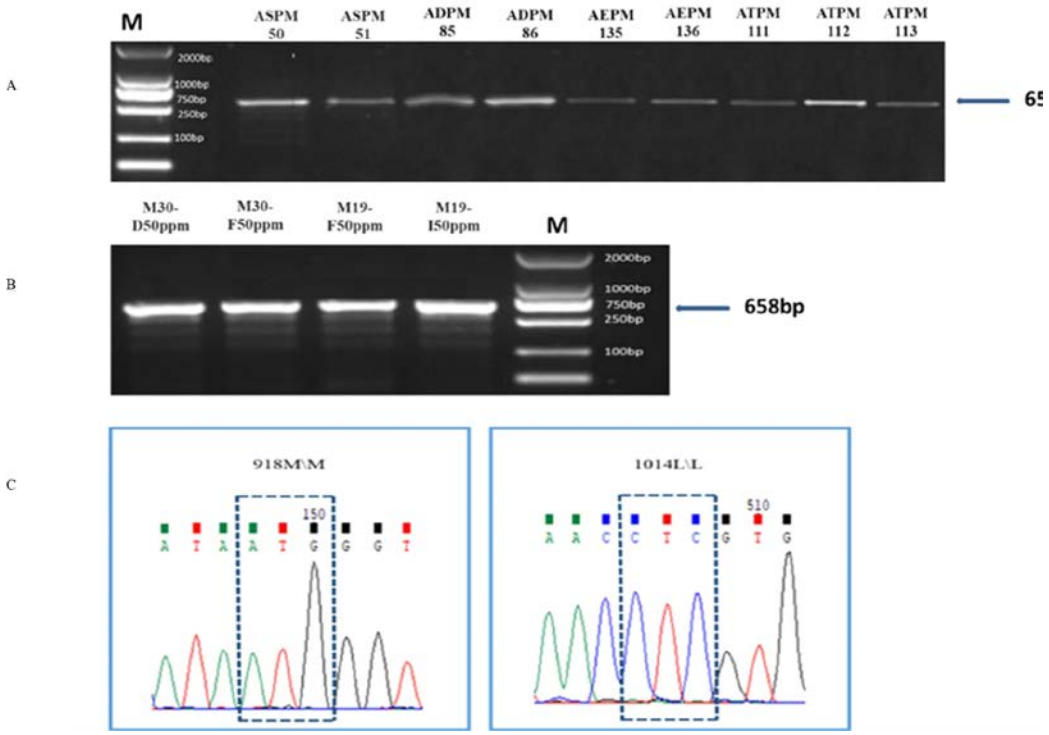

Figure 5. Detection of genetic mutations in VGSC. A. Electrophoresis detection of PCR products from representative individuals from field collections. B. Electrophoresis detection of aphids used in bioassay experiments. C. DNA sequencing Chromatograms. Codons 918 and 1014 are dashed boxed. M: DNA marker

The two codons that are disrupted by a 64 bp intron are placed in different exons (Figure 6). Alignment analysis of a region with 540 bp in length revealed eight single nucleotide polymorphic sites in four identified haplotypes. Missense mutations were not detected.

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H1          ctaaaccaatttttttgaaatgttcaactatagCTTCGAGTATTTAAGTTGGCAAAATCTT
H2          ctaaaccaatttttttgaaatgttcaactatagCTTCGAGTATTTAAGTTGGCAAAATCTT
H3          ctaaaccaatttttttgaaatgttcaactatagCTTCGAGTATTTAAGTTGGCAAAATCTT
H4          ctaaaccaatttttttgaaatgttcaactatagCTTCGAGTATTTAAGTTGGCAAAATCTT
            ************************************************************
H1          GGCCAACATTGAATCTCTTAATATCCATAATGGGTCGAACCATCGGTGCTTTGGGTAACC
H2          GGCCAACATTGAATCTCTTAATATCCATAATGGGTCGAACCATCGGTGCTTTGGGTAACC
H3          GGCCAACATTGAATCTCTTAATATCCATAATGGGTCGAACCATCGGTGCTTTGGGTAACC
H4          GGCCAACATTGAATCTCTTAATATCCATAATGGGTCGAACCATCGGTGCTTTGGGTAACC
            ************************************************************
H1          TAACGTTTGTGTTATGCATAATCATATTTATATTCGCCGTTATGGGTATGCAGTTATTTG
H2          TAACGTTTGTGTTATGCATAATCATATTTATATTCGCCGTTATGGGTATGCAGTTATTTG
H3          TAACGTTTGTGTTATGCATAATCATATTTATATTCGCCGTTATGGGTATGCAGTTATTTG
H4          TAACGTTTGTGTTATGCATAATCATATTTATATTCGCCGTTATGGGTATGCAGTTATTTG
            ************************************************************
H1          GAAAAAACTACACAGgtaccgtataatattttcaagcttacaaatgaaaaaacacaaatg
H2          GAAAAAACTACACAGgtaccgtataatattttcaagcttacaaatgaaaaaacacaaatg
H3          GAAAAAACTACACAGgtaccgtataatattttcaagcttacaaataaaaaaacacaaatg
H4          GAAAAAACTACACAGgtaccgtataatattttcaagcttacaaatgaaaaaacacaaatg
            ********************************************** **************
H1          aaattatatttttttacagAAAAAATGTACTTGTTCAAAGACCATGAGCTTCCCCGGTGG
H2          aaattatatttttttacagAAAAAATGTACTTGTTCAAAGACCATGAGCTTCCCCGGTGG
H3          aaattatatttttttacagAAAAAATGTACTTGTTCAAAGACCATGAGCTTCCCCGGTGG
H4          aaattatatttttttacagAAAAAATGTACTTGTTCAAAGACCATGAGCTTCCCCGGTGG
            ************************************************************
H1          AACTTCACCGATTTTTTGCACTCGTTTATGATAGTATTTCGCGTATTATGTGGCGAATGG
H2          AACTTCACCGATTTTTTGCACTCGTTTATGATAGTATTTCGCGTATTATGTGGCGAATGG
H3          AACTTCACCGATTTTTTGCACTCATTTATGATAGTATTTCGCGTATTATGTGGCGAATGG
H4          AACTTCACCGATTTTTTGCACTCGTTTATGATAGTATTTCGCGTATTATGTGGCGAATGG
            ********************** ***********************************
H3          ATCGAATCAATGTGGGACTGTTTACACGTTGGAGAACCAACGTGTATACCATTCTTCTTG
H1          ATCGAATCAATGTGGGACTGTTTACACGTTGGAGAACCAACGTGTATACCATTCTTCTTG
H2          ATCGAATCAATGTGGGACTGTTTACACGTTGGAGAACCAACGTGTATACCATTCTTCTTG
H4          ATCGAATCAATGTGGGACTGTTTACACGTTGGAGAACCAACGTGTATACCATTCTTCTTG
            ************************************************************
H1          GCTACCGTTGTCATCGGTAACCTCGTGgtaaatataagtactacttatacataaatatta
H2          GCTACCGTTGTCATCGGTAACCTCGTGgtaaatataagtactatttatacataaatatta
H3          GCTACCGTTGTCATCGGTAACCTCGTGgtaaatataagtactatttatacataaatatta
H4          GCTACCGTTGTCATCGGTAACCTCGTGgtaaatataagtactatttatacataaatatta
            ******************************************* ****************
H1          taatatatgtactacttagtgcaagaatttaggagagcggtggaatgccattggggttgg
H2          tagtatatgtactactcagtgcaagaatttaggagggcggtagaatgccattggggttgg
H3          taatatatgtactactcagtgcaagaatttaggagggcggtggaatgccattggggttgg
H4          taatatatgtactactcagtgcaagaatttagaagggcggtggaatgccattggggttgg
            ** ************ ************** ** ***** ******************
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Figure 6. Alignment of nucleotide sequences of VGSC haplotypes. The 5' and 3' regions that are identical are not presented in the figure. The intron sequence is indicated in lowercase letters. The codons encoding residue 918 are underlined. Asterisks indicate identical nucleotides

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Wheat is the dominant crop in countries with a dry and mild temperature [11]. In Mongolia, wheat production had reached 412 thousand tonnes in 2019 [16]. However, pest infestation may jeopardize the sustainability of wheat production. This survey reported that the occurrence of the grain aphids in northern Mongolia was low or medium as of 2019 (Figure 1 and 2).

Because of the quantity of untreated alternative hosts that act as susceptible refuges and the comparatively low selection pressure exerted by a limited number of insecticide applications in the field, cereal aphids have long been thought to be at minimal danger of developing resistance [17]. However, the emergence of *S. avenae* populations, exhibiting insecticide resistance in England, Ireland and China [2], [4], [7]–[10] call for regular monitoring of the status of insecticide resistance in regions with the continued use of insecticides. Our bioassay showed that grain aphids in northern Mongolia were susceptible to the tested three insecticides, with a mortality rate of 84-100% at a concentration of 0.005%. Being consistent with the bioassay data, neither *kdr* nor super *kdr* mutation was observed in the samples of grain aphids collected from northern Mongolia (Table 1, Figure 5). Although insecticide resistance is not a current problem for the control of *S. avenae* in Mongolia, considering the fact that the development of insecticide resistance in *S. avenae* represents a significant threat to cereal production in the United Kingdom [8] and China [5], it is required to regularly monitor the status of insecticide resistance due to the continued use of insecticides.

CONCLUSIONS

In conclusion, we found that the occurrence of *S. avenae* was low to medium level in the agricultural zones of northern Mongolia in 2019. *S. avenae* populations in these regions were susceptible to deltamethrin, fenvalerate, and imidacloprid. Neither kdr nor super-kdr mutations were found.

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